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(54) Title: EXPRESSION PROFILE OF PROSTATE CANCER

(57) Abstract: The present invention relates to compositions and methods for cancer diagnostics, including but not limited to, cancer markers. In particular, the present invention provides gene expression profiles associated with prostate cancers. Genes identified as cancer markers using the methods of the present invention find use in the diagnosis and characterization of prostate cancer. In addition, the genes provide targets for cancer drug screens and therapeutic applications.

EXPRESSION PROFILE OF PROSTATE CANCER

This application claims priority to U.S. Patent application, serial number not yet assigned, filed with express mail label EV092300383US on 8/01/02, U.S. Provisional Application Serial no. 60/309,581 filed 8/02/01 and U.S. Provisional Application Serial no. 60/334,468 filed 11/15/01. This invention was made with government support under Grant No. 5 P50 CA69568 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to compositions and methods for cancer diagnostics, including but not limited to, cancer markers. In particular, the present invention provides gene expression profiles associated with prostate cancers. The present invention further provides novel markers useful for the diagnosis, characterization, and treatment of prostate cancers.

BACKGROUND OF THE INVENTION

Afflicting one out of nine men over age 65, prostate cancer (PCA) is a leading cause of male cancer-related death, second only to lung cancer (Abate-Shen and Shen, Genes Dev 14:2410 [2000]; Ruijter *et al.*, Endocr Rev, 20:22 [1999]). The American Cancer Society estimates that about 184,500 American men will be diagnosed with prostate cancer and 39,200 will die in 2001.

Prostate cancer is typically diagnosed with a digital rectal exam and/or prostate specific antigen (PSA) screening. An elevated serum PSA level can indicate the presence of PCA. PSA is used as a marker for prostate cancer because it is secreted only by prostate cells. A healthy prostate will produce a stable amount -- typically below 4 nanograms per milliliter, or a PSA reading of "4" or less -- whereas cancer cells produce escalating amounts that correspond with the severity of the cancer. A level between 4 and 10 may raise a doctor's suspicion that a patient has prostate cancer, while amounts above 50 may show that the tumor has spread elsewhere in the body.

When PSA or digital tests indicate a strong likelihood that cancer is present, a transrectal ultrasound (TRUS) is used to map the prostate and show any suspicious areas.

Biopsies of various sectors of the prostate are used to determine if prostate cancer is present. Treatment options depend on the stage of the cancer. Men with a 10-year life expectancy or less who have a low Gleason number and whose tumor has not spread beyond the prostate are often treated with watchful waiting (no treatment). Treatment options for more aggressive cancers include surgical treatments such as radical prostatectomy (RP), in which the prostate is completely removed (with or without nerve sparing techniques) and radiation, applied through an external beam that directs the dose to the prostate from outside the body or via low-dose radioactive seeds that are implanted within the prostate to kill cancer cells locally. Anti-androgen hormone therapy is also used, alone or in conjunction with surgery or radiation. Hormone therapy uses luteinizing hormone-releasing hormones (LH-RH) analogs, which block the pituitary from producing hormones that stimulate testosterone production. Patients must have injections of LH-RH analogs for the rest of their lives.

While surgical and hormonal treatments are often effective for localized PCA, advanced disease remains essentially incurable. Androgen ablation is the most common therapy for advanced PCA, leading to massive apoptosis of androgen-dependent malignant cells and temporary tumor regression. In most cases, however, the tumor reemerges with a vengeance and can proliferate independent of androgen signals.

The advent of prostate specific antigen (PSA) screening has led to earlier detection of PCA and significantly reduced PCA-associated fatalities. However, the impact of PSA screening on cancer-specific mortality is still unknown pending the results of prospective randomized screening studies (Etzioni *et al.*, J. Natl. Cancer Inst., 91:1033 [1999]; Maattanen *et al.*, Br. J. Cancer 79:1210 [1999]; Schroder *et al.*, J. Natl. Cancer Inst., 90:1817 [1998]). A major limitation of the serum PSA test is a lack of prostate cancer sensitivity and specificity especially in the intermediate range of PSA detection (4-10 ng/ml). Elevated serum PSA levels are often detected in patients with non-malignant conditions such as benign prostatic hyperplasia (BPH) and prostatitis, and provide little information about the aggressiveness of the cancer detected. Coincident with increased serum PSA testing, there has been a dramatic increase in the number of prostate needle biopsies performed (Jacobsen *et al.*, JAMA 274:1445 [1995]). This has resulted in a surge of equivocal prostate needle biopsies (Epstein and Potter J. Urol.,

166:402 [2001]). Thus, development of additional serum and tissue biomarkers to supplement PSA screening is needed.

SUMMARY OF THE INVENTION

The present invention relates to compositions and methods for cancer diagnostics, including but not limited to, cancer markers. In particular, the present invention provides gene expression profiles associated with prostate cancers. The present invention further provides novel markers useful for the diagnosis, characterization, and treatment of prostate cancers.

In some embodiments, the present invention provides a method for characterizing prostate tissue in a subject, comprising: providing a prostate tissue sample from a subject; and detecting the presence or absence of expression of hepsin in the sample, thereby characterizing the prostate tissue sample. In some embodiments, detecting the presence of expression of hepsin comprises detecting the presence of hepsin mRNA. In other embodiments, detecting the presence of expression of hepsin mRNA comprises exposing the hepsin mRNA to a nucleic acid probe complementary to the hepsin mRNA. In yet other embodiments, detecting the presence of expression of hepsin comprises detecting the presence of a hepsin polypeptide. In some embodiments, detecting the presence of a hepsin polypeptide comprises exposing the hepsin polypeptide to an antibody specific to the hepsin polypeptide and detecting the binding of the antibody to the hepsin polypeptide. In some embodiments, the subject comprises a human subject. In some embodiments, the sample comprises tumor tissue. In some embodiments, the tumor tissue sample is a post-surgical tumor tissue sample and the method further comprises the step of c) identifying a risk of prostate specific antigen failure based on detecting the presence or absence of expression of hepsin. In some embodiments, characterizing prostate tissue comprises identifying a stage of prostate cancer in the tissue. In some embodiments, the stage includes but is not limited to, high-grade prostatic intraepithelial neoplasia, benign prostatic hyperplasia, prostate carcinoma, and metastatic prostate carcinoma. In some embodiments, the method further comprising the step of c) providing a prognosis to the subject. In some embodiments, the prognosis comprises a

risk of developing prostate specific antigen failure. In other embodiments, the prognosis comprises a risk of developing prostate cancer.

The present invention also provides a method for characterizing prostate tissue in a subject, comprising: providing a prostate tissue sample from a subject; and detecting the presence or absence of expression of pim-1 in the sample, thereby characterizing the prostate tissue sample. In some embodiments, detecting the presence of expression of pim-1 comprises detecting the presence of pim-1 mRNA. In other embodiments, detecting the presence of expression of pim-1 mRNA comprises exposing the pim-1 mRNA to a nucleic acid probe complementary to the pim-1 mRNA. In yet other embodiments, detecting the presence of expression of pim-1 comprises detecting the presence of a pim-1 polypeptide. In some embodiments, detecting the presence of a pim-1 polypeptide comprises exposing the pim-1 polypeptide to an antibody specific to the pim-1 polypeptide and detecting the binding of the antibody to the pim-1 polypeptide. In some embodiments, the subject comprises a human subject. In some embodiments, the sample comprises tumor tissue. In some embodiments, the tumor tissue sample is a post-surgical tumor tissue sample and the method further comprises the step of c) identifying a risk of prostate specific antigen failure based on detecting the presence or absence of expression of pim-1. In some embodiments, characterizing prostate tissue comprises identifying a stage of prostate cancer in the tissue. In some embodiments, the stage includes but is not limited to, high-grade prostatic intraepithelial neoplasia, benign prostatic hyperplasia, prostate carcinoma, and metastatic prostate carcinoma. In some embodiments, the method further comprising the step of c) providing a prognosis to the subject. In some embodiments, the prognosis comprises a risk of developing prostate specific antigen failure. In other embodiments, the prognosis comprises a risk of developing prostate cancer.

The present invention further provides a method for characterizing prostate tissue in a subject, comprising: providing a prostate tissue sample; and detecting a decreased or increased expression relative to a non-cancerous prostate tissue control of two or more markers selected from the group consisting of HEPSIN, FKBP5, FASN, FOLH1, TNFSF10, PCM1, S100A11, IGFBP3, SLUG, GSTM3, IL1R2, ITGB4, CCND2, EDNRB, APP, THROMBOSPONDIN 1, ANNEXIN A1, EPHA1, NCK1, MAPK6,

SGK, HEVIN, MEIS2, MYLK, FZD7, CAVEOLIN 2, TACC1, ARHB, PSG9, GSTM1, KERATIN 5, TIMP2, GELSOLIN, ITM2C, GSTM5, VINCULIN, FHL1, GSTP1, MEIS1, ETS2, PPP2CB, CATHEPSIN B, COL1A2, RIG, VIMENTIN, MOESIN, MCAM, FIBRONECTIN 1, NBL1, ANNEXIN A4, ANEXIN A11, IL1R1, IGFBP5, CYSTATIN C, COL15A1, ADAMTS1, SKI, EGR1, FOSB, CFLAR, JUN, YWHAB, NRAS, C7, SCYA2, ITGA1, LUMICAN, C1S, C4BPA, COL3A1, FAT, MMECD10, CLUSTERIN, PLA2G2A, thereby characterizing the prostate tissue sample. In some embodiments, the detecting comprises detecting three or more markers. In other embodiments, the detecting comprises detecting five or more markers. In still further embodiments, the detecting comprises detecting ten or more markers.

The present invention additionally provides a method for characterizing prostate cancer in a subject, comprising: providing a tumor sample from a subject diagnosed with prostate cancer; and detecting decreased expression relative to a non-cancerous prostate tissue control of two or more cancer markers selected from the group consisting of IGFBP5, MADH4, NBL1, SEPP1, RAB2, FAT, PP1CB, MPDZ, PRKCL2, ATF2, RAB5A, and Cathepsin H, wherein decreased expression is diagnostic of metastatic prostate cancer. In some embodiments, the detecting comprises detecting three or more markers. In other embodiments, the detecting comprises detecting five or more markers. In still further embodiments, the detecting comprises detecting ten or more markers.

The present invention further provides a method for characterizing prostate cancer in a subject, comprising providing a tumor sample from a subject diagnosed with prostate cancer; and detecting increased expression relative to a non-cancerous prostate tissue of two or more cancer markers selected from the group consisting of CTBP1, MAP3K10, TBXA2R, MTA1, RAP2, TRAP1, TFCP2, E2-EPF, UBCH10, TASTIN, EZH2, FLS353, MYBL2, LIMK1, TRAF4, wherein increased expression is diagnostic of metastatic prostate cancer. In some embodiments, the detecting comprises detecting three or more markers. In other embodiments, the detecting comprises detecting five or more markers. In still further embodiments, the detecting comprises detecting ten or more markers.

In some embodiments, the present invention provides a kit for characterizing prostate cancer in a subject, comprising: a reagent capable of specifically detecting the presence of absence of expression of hepsin; and instructions for using the kit for

characterizing cancer in the subject. In some embodiments, the reagent comprises a nucleic acid probe complementary to a hepsin mRNA. In other embodiments, the reagent comprises an antibody that specifically binds to a hepsin polypeptide. In some embodiments, the instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

In other embodiments, the present invention provides a kit for characterizing prostate cancer in a subject, comprising: a reagent capable of specifically detecting the presence of absence of expression of pim-1; and instructions for using the kit for characterizing cancer in the subject. In some embodiments, the reagent comprises a nucleic acid probe complementary to a pim-1 mRNA. In other embodiments, the reagent comprises an antibody that specifically binds to a pim-1 polypeptide. In some embodiments, the instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

In still further embodiments, the present invention provides a kit for characterizing prostate cancer in a subject, comprising: two or more reagents capable of specifically detecting expression levels of two or more markers selected from the group consisting of FKBP5, FASN, FOLH1, TNFSF10, PCM1, S100A11, IGFBP3, SLUG, GSTM3, ATF2, RAB5A, IL1R2, ITGB4, CCND2, EDNRB, APP, THROMBOSPONDIN 1, ANNEXIN A1, EPHA1, NCK1, MAPK6, SGK, HEVIN, MEIS2, MYLK, FZD7, CAVEOLIN 2, TACC1, ARHB, PSG9, GSTM1, KERATIN 5, TIMP2, GELSOLIN, ITM2C, GSTM5, VINCULIN, FHL1, GSTP1, MEIS1, ETS2, PPP2CB, CATHEPSIN B, CATHEPSIN H, COL1A2, RIG, VIMENTIN, MOESIN, MCAM, FIBRONECTIN 1, NBL1, ANNEXIN A4, ANEXIN A11, IL1R1, IGFBP5, CYSTATIN C, COL15A1, ADAMTS1, SKI, EGR1, FOSB, CFLAR, JUN, YWHAB, NRAS, C7, SCYA2, ITGA1, LUMICAN, C1S, C4BPA, COL3A1, FAT, MMECD10, CLUSTERIN, PLA2G2A, MADh4, SEPP1, RAB2, PP1CB, MPDZ, PRKCL2, CTBP1, CTBP2, MAP3K10, TBXA2F, MTA1, RAP2, TRAP1, TFCP2, E2EPF, UBCH10, TASTIN, EZH2, FLS353, MYBL2, LIMK1, GP73, VAV2, TOP2A, ASNS, CTBP, AMACR, ABCC5 (MDR5), and TRAF4; and instructions for using the kit for characterizing cancer in the subject. In some embodiments, the kit comprises reagents capable of specifically detecting expression levels of three or more of the markers. In

other embodiments, the kit comprises reagents capable of specifically detecting expression levels of five or more of the markers. In still further embodiments, the kit comprises reagents capable of specifically detecting expression levels of ten or more of the markers. In some embodiments, the instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

In yet other embodiments, the present invention provides a kit for characterizing prostate cancer in a subject, comprising: two or more reagents capable of specifically detecting decreased expression levels of two or more markers selected from the group consisting of IGFBP5, MADH4, NBL1, SEPP1, RAB2, FAT, PP1CB, MPDZ, PRKCL2, ATF2, RAB5A, and Cathepsin H; and instructions for using the kit for characterizing cancer in the subject. In some embodiments, the kit comprises reagents capable of specifically detecting decreased expression levels of three or more of the markers. In other embodiments, the kit comprises reagents capable of specifically detecting decreased expression levels of five or more of the markers. In still further embodiments, the kit comprises reagents capable of specifically detecting decreased expression levels of ten or more of the markers. In some embodiments, the instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

In an additional embodiment, the present invention provides a kit for characterizing prostate cancer in a subject, comprising: two or more reagents capable of specifically detecting increased expression levels of two or more markers selected from the group consisting of CTBP1, MAP3K10, TBXA2R, MTA1, RAP2, TRAP1, TFCP2, E2-EPF, UBCH10, TASTIN, EZH2, FLS353, MYBL2, LIMK1, TRAF4; and instructions for using the kit for characterizing cancer in the subject. In some embodiments, the kit comprises reagents capable of specifically detecting decreased expression levels of three or more of the markers. In other embodiments, the kit comprises reagents capable of specifically detecting decreased expression levels of five or more of the markers. In still further embodiments, the kit comprises reagents capable of specifically detecting decreased expression levels of ten or more of the markers. In some embodiments, the instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

The present invention also provides a method of screening compounds, comprising: providing a prostate cell sample; and one or more test compounds; and contacting the prostate cell sample with the test compound; and detecting a change in hepsin expression in the prostate cell sample in the presence of the test compound relative to the absence of the test compound. In some embodiments, the detecting comprises detecting hepsin mRNA. In other embodiments, the detecting comprises detecting hepsin polypeptide. In some embodiments, the cell is *in vitro*. In other embodiments, the cell is *in vivo*. In some embodiments, the test compound comprises an antisense compound. In other embodiments, the test compound comprises a drug.

The present invention further provides a method of screening compounds, comprising: providing a prostate cell sample; and one or more test compounds; and contacting the prostate cell sample with the test compound; and detecting a change in pim-1 expression in the prostate cell sample in the presence of the test compound relative to the absence of the test compound. In some embodiments, the detecting comprises detecting pim-1 mRNA. In other embodiments, the detecting comprises detecting pim-1 polypeptide. In some embodiments, the cell is *in vitro*. In other embodiments, the cell is *in vivo*. In some embodiments, the test compound comprises an antisense compound. In other embodiments, the test compound comprises a drug.

The present invention provides a prostate cancer expression profile map comprising gene expression level information for two or more markers selected from the group consisting of: FKBP5, FASN, FOLH1, TNFSF10, PCM1, S100A11, IGFBP3, SLUG, GSTM3, ATF2, RAB5A, IL1R2, ITGB4, CCND2, EDNRB, APP, THROMBOSPONDIN 1, ANNEXIN A1, EPHA1, NCK1, MAPK6, SGK, HEVIN, MEIS2, MYLK, FZD7, CAVEOLIN 2, TACC1, ARHB, PSG9, GSTM1, KERATIN 5, TIMP2, GELSOLIN, ITM2C, GSTM5, VINCULIN, FHL1, GSTP1, MEIS1, ETS2, PPP2CB, CATHEPSIN B, CATHEPSIN H, COL1A2, RIG, VIMENTIN, MOESIN, MCAM, FIBRONECTIN 1, NBL1, ANNEXIN A4, ANEXIN A11, IL1R1, IGFBP5, CYSTATIN C, COL15A1, ADAMTS1, SKI, EGR1, FOSB, CFLAR, JUN, YWHAB, NRAS, C7, SCYA2, ITGA1, LUMICAN, C1S, C4BPA, COL3A1, FAT, MMECD10, CLUSTERIN, PLA2G2A, MADh4, SEPP1, RAB2, PP1CB, MPDZ, PRKCL2, CTBP1, CTBP2, MAP3K10, TBXA2F, MTA1, RAP2, TRAP1, TFCP2, E2EPF, UBCH10,

TASTIN, EZH2, FLS353, MYBL2, LIMK1, GP73, VAV2, TOP2A, ASNS, CTBP, AMACR, ABCC5 (MDR5), and TRAF4. In some embodiments, the map is digital information stored in computer memory. In some embodiments, the map comprises information for three or more markers. In other embodiments, the map comprises information for five or more markers. In still further embodiments, the map comprises information for ten or more markers.

The present invention also provides a prostate cancer expression profile map comprising gene expression level information for two or more markers selected from the group consisting of: IGFBP5, MADH4, NBL1, SEPP1, RAB2, FAT, PP1CB, MPDZ, PRKCL2, ATF2, RAB5A, and Cathepsin H. In some embodiments, the map is digital information stored in computer memory. In some embodiments, the map comprises information for three or more markers. In other embodiments, the map comprises information for five or more markers. In still further embodiments, the map comprises information for ten or more markers. In some embodiments, the prostate cancer is metastatic.

The present invention further provides a prostate cancer expression profile map comprising gene expression level information for two or more markers selected from the group consisting of: CTBP1, MAP3K10, TBXA2R, MTA1, RAP2, TRAP1, TFCP2, E2-EPF, UBCH10, TASTIN, EZH2, FLS353, MYBL2, LIMK1, TRAF4. In some embodiments, the map is digital information stored in computer memory. In some embodiments, the map comprises information for three or more markers. In other embodiments, the map comprises information for five or more markers. In still further embodiments, the map comprises information for ten or more markers. In some embodiments, the prostate cancer is metastatic.

In some embodiments, the present invention provides a method for characterizing prostate tissue in a subject, comprising providing a prostate tissue sample from a subject; and detecting the presence or absence of expression of EZH2 in the sample, thereby characterizing the prostate tissue sample. In some embodiments, detecting the presence of expression of EZH2 comprises detecting the presence of EZH2 mRNA (e.g., including, but not limited to, by exposing the hepsin mRNA to a nucleic acid probe complementary to the hepsin mRNA). In other embodiments, detecting the presence of

expression of EZH2 comprises detecting the presence of a EZH2 polypeptide (e.g., including, but not limited to, by exposing the EZH2 polypeptide to an antibody specific to the EZH2 polypeptide and detecting the binding of the antibody to the EZH2 polypeptide). In some embodiments, the subject comprises a human subject. In some embodiments, the sample comprises tumor tissue. In some embodiments, characterizing the prostate tissue comprises identifying a stage of prostate cancer in the prostate tissue. In certain embodiments, the stage is selected from the group including, but not limited to, high-grade prostatic intraepithelial neoplasia, benign prostatic hyperplasia, prostate carcinoma, and metastatic prostate carcinoma. In some embodiments, the method further comprises the step of providing a prognosis to the subject (e.g., a risk of developing metastatic prostate cancer).

In further embodiments, the present invention provides a kit for characterizing prostate cancer in a subject, comprising a reagent capable of specifically detecting the presence of absence of expression of EZH2; and instructions for using the kit for characterizing cancer in the subject. In some embodiments, the reagent comprises a nucleic acid probe complementary to a EZH2 mRNA. In other embodiments, the reagent comprises an antibody that specifically binds to a EZH2 polypeptide. In certain embodiments, the instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

In still other embodiments, the present invention provides a method of screening compounds, comprising providing a prostate cell sample; and one or more test compounds; and contacting the prostate cell sample with the test compound; and detecting a change in EZH2 expression in the prostate cell sample in the presence of the test compound relative to the absence of the test compound. In some embodiments, wherein the detecting comprises detecting EZH2 mRNA. In other embodiments, the detecting comprises detecting EZH2 polypeptide. In some embodiments, the cell is *in vitro*; while in other embodiments, the cell is *in vivo*. In some embodiments, the test compound comprises an antisense compound. In certain embodiments, the test compound comprises a drug.

In yet other embodiments, the present invention provides a method for characterizing inconclusive prostate biopsy tissue in a subject, comprising providing an

inconclusive prostate biopsy tissue sample from a subject; and detecting the presence of expression of AMACR in the sample, thereby characterizing the inconclusive prostate biopsy tissue sample. In some embodiments, detecting the presence of expression of AMACR comprises detecting the presence of AMACR mRNA (e.g., by exposing the AMACR mRNA to a nucleic acid probe complementary to at least a portion of the AMACR mRNA). In other embodiments, detecting the presence of expression of AMACR comprises detecting the presence of a AMACR polypeptide (e.g., by exposing the AMACR polypeptide to an antibody specific to the AMACR polypeptide and detecting the binding of the antibody to the AMACR polypeptide). In some embodiments, the subject comprises a human subject. In some embodiments, the presence of AMACR expression in the inconclusive biopsy tissue is indicative of prostate cancer in the subject. In certain embodiments, the method further comprises the step of detecting expression of a basal cell marker selected from the group consisting of 34βE12 and p63 and the absence of a basal cell marker expression and the presence of AMACR expression is indicative of prostate cancer in the subject.

The present invention further provides a method of detecting AMACR expression in a bodily fluid, comprising providing a bodily fluid from a subject; and a reagent for detecting AMACR expression in the biological fluid; and contacting the bodily fluid with the reagent under conditions such that the reagent detects AMACR expression in the bodily fluid. In some embodiments, the bodily fluid is selected from the group consisting of serum, urine, whole blood, lymph fluid, and mucus. In certain embodiments, the presence of AMACR in the bodily fluid is indicative of cancer (e.g., prostate cancer).

The present invention additionally provides a kit for characterizing inconclusive prostate biopsy tissue in a subject, comprising a reagent capable of specifically detecting the presence or absence of expression of AMACR; and instructions for using the kit for characterizing inconclusive biopsy tissue in the subject. In some embodiments, the reagent comprises a nucleic acid probe complementary to at least a portion of an AMACR mRNA. In other embodiments, the reagent comprises an antibody that specifically binds to a AMACR polypeptide. In still other embodiments, the kit further comprises a second reagent, the second reagent capable of specifically detecting the expression of a basal cell marker selected from the group consisting of 34βE12 and p63.

In some embodiments, the instructions further comprise instructions for using the second reagent and the reagent for characterizing inconclusive biopsy tissue in the subject. In some embodiments, the instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

The present invention further provides a method of characterizing tissue in a subject, comprising providing a tissue sample from a subject, the tissue sample selected from the group consisting of breast tissue, ovarian tissue, lymph tissue, and melanoma tissue; and detecting the presence or absence of expression of AMACR in the sample, thereby characterizing the breast tissue sample.

The present invention also provides a method of diagnosing cancer in a subject, comprising providing a tissue sample from a subject, the tissue sample selected from the group consisting of breast tissue, ovarian tissue, lymph tissue, and melanoma tissue; and wherein the subject is suspected of having cancer; and detecting the presence of expression of AMACR in the sample, thereby diagnosing cancer in the tissue sample of the subject.

The present invention provides a method of diagnosing cancer in a subject, comprising providing a blood sample from a subject suspected of having cancer; and detecting an immune response to AMACR in the blood sample, thereby diagnosing cancer in the subject. In some embodiments, the cancer is prostate cancer. In certain embodiments, detecting an immune response comprises detecting an antibody against the AMACR in the blood sample.

The present invention additionally provides a method of inhibiting the growth of cells, comprising providing a cell that expresses EZH2; and a reagent for inhibiting EZH2 expression in the cell; and contacting the cell with the reagent under conditions such that the expression of EZH2 in the cell is inhibited. In some embodiments, the reagent is an antisense oligonucleotide. In other embodiments, the reagent is a RNA duplex. In some embodiments, the reagent is a drug. In some embodiments, the cell is a prostate cancer cell. In some embodiments, the cell is *in vitro*; while in other embodiments, the cell is *in vivo*. In some embodiments, the contacting further results in a decrease in proliferation of the cell.

In certain embodiments, the present invention provides a method for characterizing prostate cancer in a subject, comprising providing a prostate tissue sample from a subject suspected of having prostate cancer; and detecting a decrease in expression of an annexin in the sample, thereby characterizing the prostate tissue sample. In some embodiments, the decrease in expression of an annexin is indicative of the presence of metastatic prostate cancer in the subject. In certain embodiments, the annexin is selected from the group including, but not limited to, Annexin 1, Annexin 2, Annexin 4, Annexin 6, Annexin 7, and Annexin 11.

In other embodiments, the present invention provides a method for characterizing prostate cancer in a subject, comprising providing a prostate tissue sample from a subject suspected of having prostate cancer; and detecting an increase in expression of a c-terminal binding protein in the sample, thereby characterizing the prostate tissue sample. In some embodiments, the c-terminal binding protein is selected from the group consisting of c-terminal binding protein 1 and c-terminal binding protein 2. In certain embodiments, the increase in expression of a c-terminal binding protein is indicative of the presence of metastatic prostate cancer in the subject. In some embodiments, the expression of a c-terminal binding protein is indicative of an increased risk of PSA failure.

In other embodiments, the present invention provides a method for characterizing prostate cancer in a subject, comprising providing a prostate tissue sample from a subject suspected of having prostate cancer; and detecting an increase or decrease in expression of GP73, thereby characterizing the prostate tissue sample. In some embodiments, an increase in expression of gp73 is indicative of localized prostate cancer. In other embodiments, the prostate tissue sample is prostate cancer and a decrease in the expression of gp73 is indicative of metastatic prostate cancer.

DESCRIPTION OF THE FIGURES

Figure 1 shows a gene expression profile of prostate cancer samples. Figure 1a shows a dendrogram describing the relatedness of the samples. Figures 1b shows a cluster diagram of the samples groups compared against normal

adjacent prostate pool as a reference. Figures 1c shows a cluster diagram of the samples groups compared against commercial prostate pool reference.

Figure 2 shows functional clusters of genes differentially expressed in prostate cancer.

Figure 3 shows the expression of hepsin in prostate cancer samples as determined by Northern blot analysis and immunohistochemistry. Figure 3a shows Northern blot analysis of human hepsin (top) and normalization with GAPDH (bottom). NAT indicates normal adjacent prostate tissue and PCA indicates prostate cancer. Figure 3b shows tissue microarrays used for hepsin analysis. Figure 3c shows a histogram of hepsin protein expression by tissue type. Benign prostate hyperplasia (BPH). High-grade intraepithelial neoplasia (HG-PIN). Localized prostate cancer (PCA). Hormone-refractory prostate cancer (MET). Figure 3d shows Kaplan Meier Analysis.

Figure 4 shows the expression of pim-1 in prostate cancer samples as determined by Northern blot analysis and immunohistochemistry. Figure 4a shows a histogram of pim-1 protein expression by tissue type as assessed from 810 tissue microarray elements. High-grade intraepithelial neoplasia (HG-PIN). Localized prostate cancer (PCA). Figure 4b shows a Kaplan-Meier analysis. The tope line represents patients with strong Pim-1 staining. The bottom line represents patients with absent/weak Pim-1 expression.

Figure 5 shows a comparison of gene expression profiles for normal adjacent prostate tissue and normal prostate tissue reference.

Figure 6 shows a focused cluster of prostate cancer related genes.

Figure 7 shows data for gene selection based on computed t-statistics for the NAP and CP pools.

Figure 8 shows an overview of genes differentially expressed in prostate cancer.

Figure 9 describes exemplary accession numbers and sequence ID Numbers for exemplary genes of the present invention.

Figure 10 provides exemplary sequences of some genes of the present invention.

Figure 11 an overview of the discovery and characterization of AMACR in prostate cancer utilized in some embodiments of the present invention.

Figure 12 describes a DNA microanalysis of AMACR expression in prostate cancer.

Figure 13 describes an analysis of AMACR transcript and protein levels in prostate cancer.

Figure 14 describes an analysis of AMACR protein expression using prostate cancer tissue microarrays.

Figure 15 shows relative gene expression of AMACR in several samples.

Figure 16 shows AMACR protein expression PCA. Figure 19A shows AMACR protein expression in localized hormone naive PCA. Figure 19B shows strong AMACR expression in a naive lymph node metastasis. Error bars represent the 95% CI of the mean expression of the primary naive prostate cancer and corresponding lymph node metastases.

Figure 17 shows the hormonal effect on AMACR expression. Figure 17A shows PCA demonstrating strong hormonal effect due to anti-androgen treatment. Figure 17B shows Western Blot analysis representing the baseline AMACR expression in different prostate cell lines (Left) and Western Blot analysis of LNCaP cells for AMACR and PSA expression after treatment with an androgen or an anti-androgen for 24h and 48 hours (right).

Figure 18 shows AMACR over-expression in multiple tumors. AMACR protein expression was evaluated by immunohistochemistry on a multi-tumor and a breast cancer tissue microarray. Percentage of cases with positive staining (moderate and strong staining intensity) is summarized on the Y-axis. The left bar represents negative or weak staining and the right bar represents moderate or strong staining.

Figure 19 shows the results of laser capture microdissection (LCM) and RT-PCR amplification of AMACR in prostate cancer. LCM was used to isolate pure prostate cancer and benign glands and AMACR gene expression was characterized by RT-PCR in 2 radical prostatectomies. A constitutively expressed gene, GAPDH, was used as quantitative control of input mRNA. AMACR expression is barely detectable in benign glands, and is elevated in prostate cancer.

Figure 20 describes the identification and validation of EZH2 over-expression in metastatic prostate cancer. Figure 20a shows a cluster diagram depicting genes that molecularly distinguish metastatic prostate cancer (MET) from clinically localized prostate cancer (PCA). Figure 20b shows a DNA microarray analysis of prostate cancer

that reveals upregulation of EZH2 in metastatic prostate cancer. Figure 20c shows RT-PCR analysis of the EZH2 transcript in prostate tissue and cell lines. Figure 20d shows increased expression of EZH2 protein in prostate cancer.

Figure 21 shows that EZH2 protein levels correlate with the lethal progression and aggressiveness of prostate cancer. Figure 21a shows tissue microarray analysis of EZH2 expression. The mean EZH2 protein expression for the indicated prostate tissues is summarized using error bars with 95% confidence intervals. Figure 21b shows a Kaplan-Meier analysis demonstrating that patients with clinically localized prostate cancers that have high EZH2 expression (Moderate/Strong staining) have a greater risk for prostate cancer recurrence after prostatectomy (log rank test, $p= 0.03$).

Figure 22 shows the role of EZH2 in prostate cell proliferation. Figure 22a shows an immunoblot analysis of RNA interference using siRNA duplexes targeting the EZH2 sequence in prostate cells. Figure 22b shows that RNA interference of EZH2 decreases cell proliferation as assessed by cell counting assay. Figure 22c shows that RNA interference of EZH2 inhibits cell proliferation as assessed by WST assay. Figure 22d shows that RNA interference of EZH2 induces G2/M arrest of prostate cells.

Figure 23 shows that EZH2 functions as a transcriptional repressor in prostate cells. Figure 23a shows a schematic diagram of EZH2 constructs used in transfection/transcriptome analysis. ER, modified ligand binding domain of estrogen receptor. H-1 and H-2, homology domains 1 and 2 which share similarity between EZH2 and E(z). CYS, cysteine-rich domain. SET, SET domain. TAG, myc-epitope tag. NLS, nuclear localization signal. Figure 23b shows confirmation of expression of EZH2 constructs used in a. An anti-myc antibody was used. Figure 23c shows a cluster diagram of genes that are significantly repressed by EZH2 overexpression. Figure 23d shows SAM analysis of gene expression profiles of EZH2 transfected cells compared against EZH2 .SET transfected cells. Figure 23e shows a model for potential functional interactions of EZH2 as elucidated by transcriptome analysis and placed in the context of previously reported interactions. +, induction. -, repression.

Figure 24 shows the detection of AMACR in PCA cell lines.

Figure 25 shows the detection of AMACR protein in serum by quantitation of microarray data.

Figure 26 shows an immunoblot analysis of serum from patients with either negative or positive PSA antigen.

Figure 27 shows an immunoblot analysis of the presence of AMACR in urine samples from patients with bladder cancer (females) or bladder cancer and increased PSA (males).

Figure 28 shows representative data of a humoral response by protein microarray analysis.

Figure 29 shows immunoblot analysis of the humoral response of AMACR. Figure 29a shows an immunoblot analysis of the humoral response to AMACR. Figure 29b shows a control experiment where the humoral response was blocked.

Figure 30 shows GP73 Transcript levels in prostate cancer. Figure 30a shows the level of GP73 in individual samples after microarray analysis. Figure 30b shows the result of GP73 transcripts determined by DNA microarray analysis from 76 prostate samples grouped according to sample type and averaged.

Figure 31 shows that GP73 protein is upregulated in prostate cancer. Figure 31a shows Western blot analysis of GP73 protein in prostate cancer. Figure 31b shows an immunoblot analysis of the Golgi resident protein Golgin 97.

Figure 32 shows immunoblot analysis of normal and prostate cancer epithelial cells.

Figure 33 shows the cDNA expression of select annexin gene family members.

Figure 34 shows a heat map representation of annexin family gene expression across four prostate cancer profiling studies. Over and under expression at the transcript level are represented by shades of red and green, respectively. Gray shading indicates that insufficient data was available. Each square represents an individual tissue sample.

Figure 35 shows the expression of CtBP proteins in PCA specimens.

Figure 36 shows tissue microarray analysis of CtBP in prostate cancer that suggests mis-localization during prostate cancer progression.

Figure 37 shows the sub-cellular fractionation of LNCaP cells.

Figure 38 shows a Kaplan-Meier Analysis of prostate cancer tissue microarray data.

GENERAL DESCRIPTION

Exploring the molecular circuitry that differentiates indolent PCA from aggressive PCA has the potential to lead to the discovery of prognostic markers and novel therapeutic targets. Insight into the mechanisms of prostate carcinogenesis is also gleaned by such a global molecular approach. Similar to breast cancer (Lopez-Otin and Diamandis, Endor. Rev., 19:365 [1998]), PCA develops in a complex milieu of genetic and environmental factors in which steroid hormone signaling plays a central role. The primary precursor lesion of PCA, high-grade prostatic intraepithelial neoplasia (HG-PIN), has several characteristics similar to other early invasive carcinomas (*i.e.*, chromosomal abnormalities and cytologic features). Loss of specific chromosomal regions (*e.g.*, 8p21, 10q, 13q, 17p) along with losses and mutations of tumor suppressor genes such as Nkx3.1, PTEN, Rb, and p53 have been implicated in the initiation and progression of prostate cancer (Abate-Shen and Shen, *supra*). With the emergence of global profiling strategies, a systematic analysis of genes involved in PCA is now possible. DNA microarray technology is revolutionizing the way fundamental biological questions are addressed in the post-genomic era. Rather than the traditional approach of focusing on one gene at a time, genomic-scale methodologies allow for a global perspective to be achieved. The power of this approach lies in its ability to comparatively analyze genome-wide patterns of mRNA expression (Brown and Botstein, Nat. Gent., 21:33 [1999]). Obtaining large-scale gene expression profiles of tumors allows for the identification of subsets of genes that function as prognostic disease markers or biologic predictors of therapeutic response (Emmert-Buck *et al.*, Am. J. Pathol., 156:1109 [2000]). Golub *et al.* used DNA arrays in the molecular classification of acute leukemias (Golub *et al.*, Science 286:531 [1999], demonstrating the feasibility of using microarrays for identifying new cancer classes (class discovery) and for assigning tumors to known classes (class prediction). Using a similar approach, Alizadeh *et al.* showed that diffuse large B-cell lymphoma could be dissected into two prognostic categories by gene expression profiling (Alizadeh *et al.*, Nature 403:503 [2000]). They provided evidence that lymphomas possessing a gene expression signature characteristic of germinal center B cells had a more favorable prognosis than those expressing genes characteristic of activated peripheral B-cells. Similar large-scale classifications of breast cancer and

melanoma have been undertaken, and as with the other studies, molecular classification was the primary focus (Alizadeh *et al.*, *supra*).

Accordingly, the present invention provides an analysis of gene expression profiles in benign and malignant prostate tissue. Three candidate genes, AMACR, hepsin and pim-1, identified by DNA microarray analysis of PCA, were characterized at the protein level using PCA tissue microarrays. Analysis of the differential gene expression profiles of normal and neoplastic prostate has led to the identification of a select set of genes that define a molecular signature for PCA. The expression profiling experiments of the present invention demonstrate a role for multiple, collaborative gene expression alterations which ultimately manifest as the neoplastic phenotype. By making direct comparative hybridizations of normal and neoplastic tissues, genes that molecularly distinguish benign tissue from malignant are identified.

α-Methylacyl-CoA Racemase (AMACR) is an enzyme that plays an important role in bile acid biosynthesis and β-oxidation of branched-chain fatty acids (Ferdinandusse *et al.*, J. Lipid Res., 41:1890 [2000]; Kotti *et al.*, J. Biol.Chem., 275:20887 [2000]). Mutations of the AMACR gene have been shown to cause adult-onset sensory motor neuropathy (Ferdinandusse *et al.*, Nat. Genet., 24:188 [2000]). In diagnostically challenging prostate biopsy cases, pathologists often employ the basal cell markers 34βE12 or p63, which stain the basal cell layer of benign glands that is not present in malignant glands. Thus, in many biopsy specimens, the pathologist must rely on absence of staining to make the final diagnosis of prostate cancer. Experiments conducted during the development of the present invention identified AMACR as a marker expressed in cancerous biopsy tissue. Thus, the clinical utility of AMACR in prostate needle biopsies is large. For example, at the University of Michigan Medical Center, approximately 400 prostate needle biopsies are performed per year and approximately 20% require the use of a basal-cell specific marker to evaluate difficult lesions, characterized by a small amount of atypical glands. Accordingly, it is contemplated that in combination with basal cell specific markers, such as 34βE12 or p63, screening for AMACR expression by the methods of the present invention results in fewer cases diagnosed as "atypical without a definitive diagnosis."

Identification of the over-expression of AMACR in prostate cancer has clinical utility beyond diagnostic uses. Experiments conducted during the development of the present invention revealed that the only non-cancerous tissue to expresses significant levels of AMACR protein is the human liver. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism in not necessary to practice the present invention. Nonetheless, it is contemplated that AMACR activity is required for prostate cancer growth and by virtue of its specificity serves as a therapeutic target.

Additional experiments conducted during the course of development of the present invention investigated AMACR expression in different groups of prostate cancer, including the aspect of neo-adjuvant hormonal withdrawal in localized disease. AMACR expression was found to be hormone independent in cell culture experiments. PSA, a gene known to be regulated by androgens, demonstrated hormone related alterations in expression under the same conditions. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that these findings provide evidence that AMACR is not regulated by the androgen pathway. It is further contemplated that the decreased AMACR expression in hormone refractory tissue allows the use of AMACR as a biomarker for hormone resistance. It is also contemplated that, given the fact that hormone treatment in the mean of hormonal withdrawal did not affect AMACR expression in the cell culture, that some other mechanism than the androgen pathway is responsible for AMACR downregulation in the integrity of cancer tissue.

The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that, alternatively, AMACR is over expressed in the development of cancer, perhaps playing an important role in providing energy for the neoplastic cells. However, as the tumors become de-differentiated, they no longer require these sources of energy. It is contemplated that poorly differentiated tumors may take over other pathways to accomplish this same activity of branched fatty acid oxidation. There is no association with the proliferative rate of the tumor cells and AMACR expression.

AMACR expression was also examined in other cancers. Examination of other tumors demonstrated that colon cancer has the highest AMACR expression. As colorectal cancers are not known to be hormonally regulated, the fact that de-differentiation and decreased AMACR expression were correlated in PCA further supports the hypothesis that de-differentiation leads to decreased AMACR expression in the hormone refractory metastatic PCA. Hormone treatment is also a front line therapy in metastatic prostate cancer but is known to loose efficacy, selecting out hormone insensitive clones. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that this phenomenon explains the observation that strong hormone treatment effect is consistent with decreased AMACR expression due to selection of potentially more de-differentiated cells.

The AMACR gene product is an enzyme, which plays an important role in bile acid biosynthesis and beta-oxidation of branched-chain fatty acids (Kotti *et al.*, J. Biol. Chem. 275:20887 [2000]; Ferdinandusse *et al.*, J Lipid Res 42:137 [2001]). AMACR over expression occurs in tumors with a high percentage of lipids such as PCA and colorectal cancer. The relationship between fatty acid consumption and cancer is a controversial subject in the development of PCA and colorectal cancer (Moyad, Curr Opin Urol 11:457 [2001]; Willett, Oncologist 5:393 [2000]). An essential role for AMACR in the oxidation of bile acid intermediates has been demonstrated. AMACR encodes an enzyme which catalyzes the racemization of alpha-methyl branched carboxylic coenzyme A thioesters and is localized in peroxisomes and mitochondria (Schmitz *et al.*, Eur J Biochem 231:815 [1995]). The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that, as AMACR is involved in the metabolism of lipids, that this leads to alterations in the oxidant balance of a cell. It is further contemplated that these changes are associated with DNA damage, malignant transformation, and other parameters of cell disturbance.

Additional experiments conducted during the course of development of the present invention demonstrated that AMACR mRNA and protein product are over expressed in a number of adenocarcinomas, including colorectal, prostate, breast, and

ovarian and melanoma. Adenocarcinoma from the colorectum and prostate demonstrated consistent AMACR over expression (92% and 83% of tumor, respectively). Thus, AMACR is of use in the diagnosis of colonic neoplasia. For example, in some embodiments of the present invention, AMACR is used in the diagnosis of dysplasia. Specifically, in the setting of inflammatory bowel disease (IBD), where the identification of dysplasia may be diagnostically challenging, one evaluates putative lesions for their AMACR protein expression intensity. In some embodiments, this is performed in conjunction with the analysis of the adenomatous polyposis coli gene, since mutations in this gene are also believed to occur early in the development of colorectal neoplasia (Kinzler and Vogelstein, Cell 87:159 [1996]; Tsao and Shibata, Am J Pathol 145: 531 [1994]).

Colonic adenomas (Kinzler and Vogelstein, *supra*; Tsao and Shibata, *supra*) and high-grade PIN (McNeal and Bostwick, Hum Pathol 17:64 [1986]; McNeal *et al.*, Lancet 1:60 [1986]) are well know precursors of invasive colonic and prostate cancer, respectively. Experiments conducted during the course of development of the present invention demonstrated that AMACR is over expressed in colorectal adenomas (75%) and high-grade PIN (64%). Further supporting AMACR expression in early neoplastic lesions was the presence of focal AMACR expression in some atrophic prostate lesions. Some atrophic lesions (*i.e.*, proliferative inflammatory atrophy and postatrophic hyperplasia) have recently been recognized as proliferative in nature with molecular alterations suggestive of early neoplastic changes (De Marzo *et al.*, Am J Pathol 155:1985 [1999]; Shah *et al.*, Am J Pathol 158:1767 [2001]). Some morphologically benign prostate glands were also observed to have focal moderate AMACR staining. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that AMACR may have a role in the early steps of cancer development.

Several cancers that are associated with AMACR over expression, including colorectal, prostate and breast cancer, have been linked to high-fat diet. The exact mechanism how high-fat diet contributes to tumorigenesis in these organ systems is unknown, but emerging evidence suggest that peroxisome proliferator activated receptor (PPAR) mediated pathway plays a critical role (Debril *et al.*, J. Mol. Med. 79:30 [2001]).

Diet fatty acids have been shown to function as peroxisome proliferators and bind to and activate PPARs (Zomer *et al.*, J. Lipid Res. 41:1801 [2000]), a family of nuclear receptor transcriptional factors. Activation of PPAR mediated pathways in turn control cell proliferation and differentiation. In addition, it can also alter the cellular oxidant balance (Yeldandi *et al.*, Mutat. Res. 448:159 [2000]). The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that these effects act in concert to contribute to the tumorigenesis of several cancers. This hypothesis is supported by the findings that peroxisome proliferators, when given to mice, enhance the development colon adenomatous polyps in mice (Saez *et al.*, Nat. Med. 4:1058 [1998]). In addition, PPARs are expressed in several prostate cancer cell lines and their ligands, and peroxisome proliferators, when added to culture, affect the growth of these cell lines (Shappell *et al.*, Cancer Res. 61:497 [2001]; Mueller *et al.*, PNAS 97:10990 [2000]). A phase II clinical trial also showed that troglitazone, a PPARγ activator, could stabilize PSA level in patients with prostate cancer (Kubota *et al.*, Cancer Res. 58:3344 [1998]; Hisatake *et al.*, Cancer Res. 60:5494 [2000]).

AMACR is an involved in the β-oxidation of pristanic acid (Ferdinandusse *et al.*, J. Lipid. Res. 41:1890 [2000]). Pristanic acid can function as a PPAR α activator and promote cell growth (Zomer *et al.*, J. Lipid Res. 41:1801 [2000]). The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that hyperfunctioning of β-oxidation pathway leads to exhaustion of reducing molecules and alters the cellular oxidant status (Yeldandi *et al.*, Mutat. Res. 448:159 [2000]).

The present invention further provides methods of targeting AMACR as a therapeutic target in cancer treatment. Over expressed in high percentage of colorectal, prostate, breast and melanoma, but not in adjacent normal tissues, AMACR is targeted using antibody or enzyme inhibitors. Toxicity is expected not to be a major concern because individuals with congenital absence of this enzyme have no or insignificant clinical manifestations (Clayton *et al.*, Biochem. Soc. Trans. 29:298 [2001]).

Experiments conducted during the course of development of the present invention further demonstrated that AMACR is present in the serum of prostate cancer patients. In

addition, a humoral response to AMACR was identified based on the presence of antibodies to AMACR in the serum of prostate cancer patients.

Annexins are a group of structurally related calcium-binding proteins, which have a domain that binds to phospholipids and an amino terminal domain that determines specificity (Smith *et al.*, Trends. Genet. 10:241 [1994]; Mailliard *et al.*, J Biol. Chem. 271:719 [1996]). The annexins are involved in regulation of membrane trafficking, cellular adhesion and possible tumorigenesis. Experiments conducted during the course of development of the present invention used cDNA microarrays to study the expression patterns of multiple annexin family members in a wide range of prostate tissue samples in order to determine their role in PCA progression. Meta-analysis of gene expression data was employed to help further validate the cDNA expression array findings. Finally, high-density tissue microarrays were used to assess annexin protein expression levels by immunohistochemistry.

Eight annexins were evaluated for their mRNA expression levels in benign prostatic tissue, localized hormone naïve PCA and metastatic hormone refractory PCA samples. Five annexins (1,2,4,7,and 11) demonstrated a progressive down regulation at the transcript level going from benign prostatic tissue to localized PCA to hormone refractory PCA. In order to validate the cDNA expression array finding of these 5 annexin family members, a meta-analysis was performed, which confirmed that when looking across 4 studies where at least two studies reported results, annexin 1,2,4, and 6 were significantly down regulated in localized PCA samples when compared to benign prostatic tissue. Therefore the meta-analysis confirmed results on annexin 1, 2, and 4. In these examples, summary statistics across all datasets found these annexins to be significantly down regulated at the cDNA level. However, not all of the 4 studies had significant down-regulation. Annexin 4, for example, was significantly down regulated in two of four studies but the resultant summary statistic, which also takes into account the number of samples evaluated, was statistically significant. Annexins 7,8, and 13 were not found to be significantly under expressed. As demonstrated in figure 1, annexin 7 does decrease significantly when comparing localized PCA and metastatic PCA.

The protein expression levels of all above five annexins tested were statistically significantly decreased in hormone refractory PCA samples when compared to either

localized PCA or benign prostate tissue. Four of 5 annexins also demonstrated a decrease in protein expression in clinically localized PCA as compared to benign prostate tissue. However, in none of these cases was the protein expression found to be significantly decreased. This second validation method at the protein level confirmed the cDNA expression array data for annexin 1,2,4, 7, and 11.

Based on gene expression array data described herein, localized PCA cells down regulate their mRNA levels of annexins but maintained the corresponding protein expression levels. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that post-translational alteration may compensate for decrease mRNA, producing enough protein to maintain levels seen with benign samples. Since annexins play an important role in maintaining cellular adhesion, once the cells eventually lose this ability, tumor progression may occur. Therefore, as one might anticipate, annexin expression levels decreased significantly in the advanced hormone refractory PCA samples. This was confirmed at the protein level by significant decreases as demonstrated by immunohistochemistry.

A sequential down-regulation of annexins in both transcriptional and translational levels in metastatic PCA samples was observed. Annexin I, also called lipocortin, has been described as a phospholipase A2 inhibitor, and served as a substrate of epidermal growth factor receptor (Pepinsky *et al.*, Nature 321:81 [1986]; Wallner *et al.*, Nature 320:77 [1986]). The significant reduction of protein level has been shown in esophageal and prostate tumor cells (Paweletz *et al.*, Cancer Res. 60:6293 [2000]). Annexin 2, also called p36, appears an efficient substrate of protein kinase C and Src pp60 (Hubaishy *et al.*, Biochemistry 34:14527 [1995]). Annexin 4, called endonexin, regulates Cl- flux by mediating calmodulin kinase II (CaMKII) activity (Chan *et al.*, J. Biol. Chem. 269:32464 [1994]). Annexin 7, synexin, is involved in Duchenne's muscular dystrophy (Selbert *et al.* Exp. Cell. Res. 222:199 [1996]). Its gene is located on human chromosome 10q21, and its protein expression was decreased in hormone refractory tumor cells. In conclusion, the results of experiments conducted during the course of development of the present invention suggest that down regulation of several annexin family members may play a role in the development of the lethal PCA phenotype.

Additional experiments conducted during the course of development of the present invention identified additional markers that exhibited altered (e.g., increased or decreased) expression in prostate cancer. Additional markers include, but are not limited to, EZH2, Annexins 1, 2, 4, 7, and 11, CTBP 1 and 2, GP73, ABCC5 (MDR5), ASNS, TOP2A, and Vav2. In particular, EZH2 was identified as a marker that was overexpressed in prostate cancer, and in particular, in metastatic prostate cancer. EZH2 was further identified as being correlated with clinical failure (e.g., increased PSA levels). In addition, siRNA inhibition of EZH2 resulted in a decrease in cell proliferation of a prostate cancer cell line.

The present invention thus identifies markers and targets for diagnostic and therapeutic agents in a variety of cancers.

DEFINITIONS

To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

The term "epitope" as used herein refers to that portion of an antigen that makes contact with a particular antibody.

When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as "antigenic determinants". An antigenic determinant may compete with the intact antigen (*i.e.*, the "immunogen" used to elicit the immune response) for binding to an antibody.

The terms "specific binding" or "specifically binding" when used in reference to the interaction of an antibody and a protein or peptide means that the interaction is dependent upon the presence of a particular structure (*i.e.*, the antigenic determinant or epitope) on the protein; in other words the antibody is recognizing and binding to a specific protein structure rather than to proteins in general. For example, if an antibody is specific for epitope "A," the presence of a protein containing epitope A (or free, unlabelled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

As used herein, the terms "non-specific binding" and "background binding" when used in reference to the interaction of an antibody and a protein or peptide refer to an interaction that is not dependent on the presence of a particular structure (*i.e.*, the antibody is binding to proteins in general rather that a particular structure such as an epitope).

As used herein, the term "subject" refers to any animal (*e.g.*, a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

As used herein, the term "subject suspected of having cancer" refers to a subject that presents one or more symptoms indicative of a cancer (*e.g.*, a noticeable lump or mass) or is being screened for a cancer (*e.g.*, during a routine physical). A subject suspected of having cancer may also have one or more risk factors. A subject suspected of having cancer has generally not been tested for cancer. However, a "subject suspected of having cancer" encompasses an individual who has received an initial diagnosis (*e.g.*, a CT scan showing a mass or increased PSA level) but for whom the stage of cancer is not known. The term further includes people who once had cancer (*e.g.*, an individual in remission).

As used herein, the term "subject at risk for cancer" refers to a subject with one or more risk factors for developing a specific cancer. Risk factors include, but are not limited to, gender, age, genetic predisposition, environmental expose, previous incidents of cancer, preexisting non-cancer diseases, and lifestyle.

As used herein, the term "characterizing cancer in subject" refers to the identification of one or more properties of a cancer sample in a subject, including but not limited to, the presence of benign, pre-cancerous or cancerous tissue, the stage of the cancer, and the subject's prognosis. Cancers may be characterized by the identification of the expression of one or more cancer marker genes, including but not limited to, the cancer markers disclosed herein.

As used herein, the term "characterizing prostate tissue in a subject" refers to the identification of one or more properties of a prostate tissue sample (*e.g.*, including but not limited to, the presence of cancerous tissue, the presence of pre-cancerous tissue that is

likely to become cancerous, and the presence of cancerous tissue that is likely to metastasize). In some embodiments, tissues are characterized by the identification of the expression of one or more cancer marker genes, including but not limited to, the cancer markers disclosed herein.

As used herein, the term "cancer marker genes" refers to a gene whose expression level, alone or in combination with other genes, is correlated with cancer or prognosis of cancer. The correlation may relate to either an increased or decreased expression of the gene. For example, the expression of the gene may be indicative of cancer, or lack of expression of the gene may be correlated with poor prognosis in a cancer patient. Cancer marker expression may be characterized using any suitable method, including but not limited to, those described in illustrative Examples 1-15 below.

As used herein, the term "a reagent that specifically detects expression levels" refers to reagents used to detect the expression of one or more genes (*e.g.*, including but not limited to, the cancer markers of the present invention). Examples of suitable reagents include but are not limited to, nucleic acid probes capable of specifically hybridizing to the gene of interest, PCR primers capable of specifically amplifying the gene of interest, and antibodies capable of specifically binding to proteins expressed by the gene of interest. Other non-limiting examples can be found in the description and examples below.

As used herein, the term "detecting a decreased or increased expression relative to non-cancerous prostate control" refers to measuring the level of expression of a gene (*e.g.*, the level of mRNA or protein) relative to the level in a non-cancerous prostate control sample. Gene expression can be measured using any suitable method, including but not limited to, those described herein.

As used herein, the term "detecting a change in gene expression (*e.g.*, hepsin, pim-1, or AMACR) in said prostate cell sample in the presence of said test compound relative to the absence of said test compound" refers to measuring an altered level of expression (*e.g.*, increased or decreased) in the presence of a test compound relative to the absence of the test compound. Gene expression can be measured using any suitable method, including but not limited to, those described in Examples 1-5 below.

As used herein, the term "instructions for using said kit for detecting cancer in said subject" includes instructions for using the reagents contained in the kit for the detection and characterization of cancer in a sample from a subject. In some embodiments, the instructions further comprise the statement of intended use required by the U.S. Food and Drug Administration (FDA) in labeling *in vitro* diagnostic products. The FDA classifies *in vitro* diagnostics as medical devices and requires that they be approved through the 510(k) procedure. Information required in an application under 510(k) includes: 1) The in vitro diagnostic product name, including the trade or proprietary name, the common or usual name, and the classification name of the device; 2) The intended use of the product; 3) The establishment registration number, if applicable, of the owner or operator submitting the 510(k) submission; the class in which the in vitro diagnostic product was placed under section 513 of the FD&C Act, if known, its appropriate panel, or, if the owner or operator determines that the device has not been classified under such section, a statement of that determination and the basis for the determination that the in vitro diagnostic product is not so classified; 4)Proposed labels, labeling and advertisements sufficient to describe the in vitro diagnostic product, its intended use, and directions for use. Where applicable, photographs or engineering drawings should be supplied; 5) A statement indicating that the device is similar to and/or different from other in vitro diagnostic products of comparable type in commercial distribution in the U.S., accompanied by data to support the statement; 6) A 510(k) summary of the safety and effectiveness data upon which the substantial equivalence determination is based; or a statement that the 510(k) safety and effectiveness information supporting the FDA finding of substantial equivalence will be made available to any person within 30 days of a written request; 7) A statement that the submitter believes, to the best of their knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted; 8) Any additional information regarding the in vitro diagnostic product requested that is necessary for the FDA to make a substantial equivalency determination. Additional information is available at the Internet web page of the U.S. FDA.

As used herein, the term "prostate cancer expression profile map" refers to a presentation of expression levels of genes in a particular type of prostate tissue (*e.g.*,

primary, metastatic, and pre-cancerous prostate tissues). The map may be presented as a graphical representation (*e.g.*, on paper or on a computer screen), a physical representation (*e.g.*, a gel or array) or a digital representation stored in computer memory. Each map corresponds to a particular type of prostate tissue (*e.g.*, primary, metastatic, and pre-cancerous) and thus provides a template for comparison to a patient sample. In preferred embodiments, maps are generated from pooled samples comprising tissue samples from a plurality of patients with the same type of tissue.

As used herein, the terms "computer memory" and "computer memory device" refer to any storage media readable by a computer processor. Examples of computer memory include, but are not limited to, RAM, ROM, computer chips, digital video disc (DVDs), compact discs (CDs), hard disk drives (HDD), and magnetic tape.

As used herein, the term "computer readable medium" refers to any device or system for storing and providing information (*e.g.*, data and instructions) to a computer processor. Examples of computer readable media include, but are not limited to, DVDs, CDs, hard disk drives, magnetic tape and servers for streaming media over networks.

As used herein, the terms "processor" and "central processing unit" or "CPU" are used interchangeably and refer to a device that is able to read a program from a computer memory (*e.g.*, ROM or other computer memory) and perform a set of steps according to the program.

As used herein, the term "stage of cancer" refers to a qualitative or quantitative assessment of the level of advancement of a cancer. Criteria used to determine the stage of a cancer include, but are not limited to, the size of the tumor, whether the tumor has spread to other parts of the body and where the cancer has spread (*e.g.*, within the same organ or region of the body or to another organ).

As used herein, the term "providing a prognosis" refers to providing information regarding the impact of the presence of cancer (*e.g.*, as determined by the diagnostic methods of the present invention) on a subject's future health (*e.g.*, expected morbidity or mortality, the likelihood of getting cancer, and the risk of metastasis).

As used herein, the term "prostate specific antigen failure" refers to the development of high prostate specific antigen levels in a patient following prostate cancer therapy (*e.g.*, surgery). See Examples 3 and 4 for examples of how prostate specific

antigen failure is determined. As used herein, the term "risk of developing prostate specific antigen failure" refers to a subject's relative risk (*e.g.*, the percent chance or a relative score) of developing prostate specific antigen failure following prostate cancer therapy.

As used herein, the term "post surgical tumor tissue" refers to cancerous tissue (*e.g.*, prostate tissue) that has been removed from a subject (*e.g.*, during surgery).

As used herein, the term "subject diagnosed with a cancer" refers to a subject who has been tested and found to have cancerous cells. The cancer may be diagnosed using any suitable method, including but not limited to, biopsy, x-ray, blood test, and the diagnostic methods of the present invention.

As used herein, the term "initial diagnosis" refers to results of initial cancer diagnosis (*e.g.* the presence or absence of cancerous cells). An initial diagnosis does not include information about the stage of the cancer of the risk of prostate specific antigen failure.

As used herein, the term "biopsy tissue" refers to a sample of tissue (*e.g.*, prostate tissue) that is removed from a subject for the purpose of determining if the sample contains cancerous tissue. In some embodiment, biopsy tissue is obtained because a subject is suspected of having cancer. The biopsy tissue is then examined (*e.g.*, by microscopy) for the presence or absence of cancer.

As used herein, the term "inconclusive biopsy tissue" refers to biopsy tissue for which histological examination has not determined the presence or absence of cancer.

As used herein, the term "basal cell marker" refers to a marker (*e.g.*, an antibody) that binds to proteins present in the basal cell layer of benign prostate glands. Exemplary basal cell markers include, but are not limited to, 34βE12 and p63 (*See e.g.*, O'Malley *et al.*, Virchows Arch. Pathol. Anat. Histopathol., 417:191 [1990]; Wojno *et al.*, Am. J. Surg. Pathol., 19:251 [1995]; Googe *et al.*, Am. J. Clin. Pathol., 107:219 [1997]; Parsons *et al.*, Urology 58:619; and Signoretti *et al.*, Am. J. Pathol., 157:1769 [2000]).

As used herein, the term "non-human animals" refers to all non-human animals including, but are not limited to, vertebrates such as rodents, non-human primates, ovines, bovines, ruminants, lagomorphs, porcines, caprines, equines, canines, felines, aves, etc.

As used herein, the term "gene transfer system" refers to any means of delivering a composition comprising a nucleic acid sequence to a cell or tissue. For example, gene transfer systems include, but are not limited to, vectors (*e.g.*, retroviral, adenoviral, adeno-associated viral, and other nucleic acid-based delivery systems), microinjection of naked nucleic acid, polymer-based delivery systems (*e.g.*, liposome-based and metallic particle-based systems), biolistic injection, and the like. As used herein, the term "viral gene transfer system" refers to gene transfer systems comprising viral elements (*e.g.*, intact viruses, modified viruses and viral components such as nucleic acids or proteins) to facilitate delivery of the sample to a desired cell or tissue. As used herein, the term "adenovirus gene transfer system" refers to gene transfer systems comprising intact or altered viruses belonging to the family Adenoviridae.

As used herein, the term "site-specific recombination target sequences" refers to nucleic acid sequences that provide recognition sequences for recombination factors and the location where recombination takes place.

As used herein, the term "nucleic acid molecule" refers to any nucleic acid containing molecule, including but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "gene" refers to a nucleic acid (*e.g.*, DNA) sequence that comprises coding sequences necessary for the production of a polypeptide, precursor, or RNA (*e.g.*, rRNA, tRNA). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (*e.g.*, enzymatic activity, ligand binding, signal transduction, immunogenicity, etc.) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and the sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb or more on either end such that the gene corresponds to the length of the full-length mRNA. Sequences located 5' of the coding region and present on the mRNA are referred to as 5' non-translated sequences. Sequences located 3' or downstream of the coding region and present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

As used herein, the term "heterologous gene" refers to a gene that is not in its natural environment. For example, a heterologous gene includes a gene from one species introduced into another species. A heterologous gene also includes a gene native to an organism that has been altered in some way (*e.g.*, mutated, added in multiple copies, linked to non-native regulatory sequences, etc). Heterologous genes are distinguished from endogenous genes in that the heterologous gene sequences are typically joined to DNA sequences that are not found naturally associated with the gene sequences in the chromosome or are associated with portions of the chromosome not found in nature (*e.g.*, genes expressed in loci where the gene is not normally expressed).

As used herein, the term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (*e.g.*, mRNA, rRNA, tRNA, or snRNA) through "transcription" of the gene (*i.e.*, via the enzymatic action of an RNA

polymerase), and for protein encoding genes, into protein through "translation" of mRNA. Gene expression can be regulated at many stages in the process. "Up-regulation" or "activation" refers to regulation that increases the production of gene expression products (*i.e.*, RNA or protein), while "down-regulation" or "repression" refers to regulation that decrease production. Molecules (*e.g.*, transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors," respectively.

In addition to containing introns, genomic forms of a gene may also include sequences located on both the 5' and 3' end of the sequences that are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5' flanking region may contain regulatory sequences such as promoters and enhancers that control or influence the transcription of the gene. The 3' flanking region may contain sequences that direct the termination of transcription, post-transcriptional cleavage and polyadenylation.

The term "wild-type" refers to a gene or gene product isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product that displays modifications in sequence and or functional properties (*i.e.*, altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally occurring mutants can be isolated; these are identified by the fact that they have altered characteristics (including altered nucleic acid sequences) when compared to the wild-type gene or gene product.

As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," and "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the amino acid sequence.

As used herein, the terms "an oligonucleotide having a nucleotide sequence encoding a gene" and "polynucleotide having a nucleotide sequence encoding a gene,"

means a nucleic acid sequence comprising the coding region of a gene or in other words the nucleic acid sequence that encodes a gene product. The coding region may be present in a cDNA, genomic DNA or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-stranded (*i.e.*, the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, etc. may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, etc. or a combination of both endogenous and exogenous control elements.

As used herein, the term "oligonucleotide," refers to a short length of single-stranded polynucleotide chain. Oligonucleotides are typically less than 200 residues long (*e.g.*, between 15 and 100), however, as used herein, the term is also intended to encompass longer polynucleotide chains. Oligonucleotides are often referred to by their length. For example a 24 residue oligonucleotide is referred to as a "24-mer". Oligonucleotides can form secondary and tertiary structures by self-hybridizing or by hybridizing to other polynucleotides. Such structures can include, but are not limited to, duplexes, hairpins, cruciforms, bends, and triplexes.

As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, for the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (*i.e.*, identity). A partially complementary sequence is a nucleic acid molecule that at least partially inhibits a completely

complementary nucleic acid molecule from hybridizing to a target nucleic acid is "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous nucleic acid molecule to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target that is substantially non-complementary (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe that can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described above.

A gene may produce multiple RNA species that are generated by differential splicing of the primary RNA transcript. cDNAs that are splice variants of the same gene will contain regions of sequence identity or complete homology (representing the presence of the same exon or portion of the same exon on both cDNAs) and regions of complete non-identity (for example, representing the presence of exon "A" on cDNA 1 wherein cDNA 2 contains exon "B" instead). Because the two cDNAs contain regions of sequence identity they will both hybridize to a probe derived from the entire gene or portions of the gene containing sequences found on both cDNAs; the two splice variants are therefore substantially homologous to such a probe and to each other.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (*i.e.*, it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. A single molecule that contains pairing of complementary nucleic acids within its structure is said to be "self-hybridized."

As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (See *e.g.*, Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization [1985]). Other references include more sophisticated computations that take structural as well as sequence characteristics into account for the calculation of T_m.

As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. Under "low stringency conditions" a nucleic acid sequence of interest will hybridize to its exact complement, sequences with single base mismatches, closely related sequences (*e.g.*, sequences with 90% or greater homology), and sequences having only partial homology (*e.g.*, sequences with 50-90% homology). Under 'medium stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, sequences with single base mismatches, and closely relation sequences (*e.g.*, 90% or greater homology). Under "high stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, and (depending on conditions such a temperature) sequences with single base mismatches. In other words, under conditions of high stringency the temperature can be raised so as to exclude hybridization to sequences with single base mismatches.

"High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharamcia), 5 g BSA (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.) (see definition above for "stringency").

"Amplification" is a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (*i.e.*, replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (*i.e.*, synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out.

Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Qβ replicase, MDV-1 RNA is the specific template for the replicase (Kacian *et al.*, Proc. Natl. Acad. Sci. USA 69:3038 [1972]). Other nucleic acids will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (Chamberlin *et al.*, Nature 228:227 [1970]). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (Wu and Wallace, Genomics 4:560 [1989]). Finally, Taq and Pfu polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences (H.A. Erlich (ed.), PCR Technology, Stockton Press [1989]).

As used herein, the term "amplifiable nucleic acid" is used in reference to nucleic acids that may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" will usually comprise "sample template."

As used herein, the term "sample template" refers to nucleic acid originating from a sample that is analyzed for the presence of "target." In contrast, "background template" is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is most often inadvertent. It may be the result

of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, that is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product that is complementary to a nucleic acid strand is induced, (*i.e.*, in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

As used herein, the term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, that is capable of hybridizing to at least a portion of another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labeled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

As used herein the term "portion" when in reference to a nucleotide sequence (as in "a portion of a given nucleotide sequence") refers to fragments of that sequence. The fragments may range in size from four nucleotides to the entire nucleotide sequence minus one nucleotide (10 nucleotides, 20, 30, 40, 50, 100, 200, etc.).

As used herein, the term "target," refers to the region of nucleic acid bounded by the primers. Thus, the "target" is sought to be sorted out from other nucleic acid sequences. A "segment" is defined as a region of nucleic acid within the target sequence.

As used herein, the term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195 4,683,202, and 4,965,188, hereby incorporated by reference, which describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified".

With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (*e.g.*, hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the

PCR process are, themselves, efficient templates for subsequent PCR amplifications.

As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

The terms "in operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one component or contaminant with which it is ordinarily associated in its natural source. Isolated nucleic acid is such present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids as nucleic acids such as DNA and RNA found in the state they exist in nature. For example, a given DNA sequence (*e.g.*, a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. However, isolated nucleic acid encoding a given protein includes, by way of example, such nucleic acid in cells ordinarily expressing the given protein where the nucleic acid is in a chromosomal

location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid, oligonucleotide, or polynucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid, oligonucleotide or polynucleotide is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand (*i.e.*, the oligonucleotide or polynucleotide may be single-stranded), but may contain both the sense and anti-sense strands (*i.e.*, the oligonucleotide or polynucleotide may be double-stranded).

As used herein, the term "purified" or "to purify" refers to the removal of components (*e.g.*, contaminants) from a sample. For example, antibodies are purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulin that does not bind to the target molecule. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind to the target molecule results in an increase in the percent of target-reactive immunoglobulins in the sample. In another example, recombinant polypeptides are expressed in bacterial host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant polypeptides is thereby increased in the sample.

"Amino acid sequence" and terms such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

The term "native protein" as used herein to indicate that a protein does not contain amino acid residues encoded by vector sequences; that is, the native protein contains only those amino acids found in the protein as it occurs in nature. A native protein may be produced by recombinant means or may be isolated from a naturally occurring source.

As used herein the term "portion" when in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid.

The term "Southern blot," refers to the analysis of DNA on agarose or acrylamide gels to fractionate the DNA according to size followed by transfer of the DNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized DNA is then probed with a labeled probe to detect DNA species complementary to the

probe used. The DNA may be cleaved with restriction enzymes prior to electrophoresis. Following electrophoresis, the DNA may be partially depurinated and denatured prior to or during transfer to the solid support. Southern blots are a standard tool of molecular biologists (J. Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, NY, pp 9.31-9.58 [1989]).

The term "Northern blot," as used herein refers to the analysis of RNA by electrophoresis of RNA on agarose gels to fractionate the RNA according to size followed by transfer of the RNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized RNA is then probed with a labeled probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists (J. Sambrook, *et al., supra,* pp 7.39-7.52 [1989]).

The term "Western blot" refers to the analysis of protein(s) (or polypeptides) immobilized onto a support such as nitrocellulose or a membrane. The proteins are run on acrylamide gels to separate the proteins, followed by transfer of the protein from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized proteins are then exposed to antibodies with reactivity against an antigen of interest. The binding of the antibodies may be detected by various methods, including the use of radiolabeled antibodies.

The term "transgene" as used herein refers to a foreign gene that is placed into an organism by, for example, introducing the foreign gene into newly fertilized eggs or early embryos. The term "foreign gene" refers to any nucleic acid (*e.g.*, gene sequence) that is introduced into the genome of an animal by experimental manipulations and may include gene sequences found in that animal so long as the introduced gene does not reside in the same location as does the naturally occurring gene.

As used herein, the term "vector" is used in reference to nucleic acid molecules that transfer DNA segment(s) from one cell to another. The term "vehicle" is sometimes used interchangeably with "vector." Vectors are often derived from plasmids, bacteriophages, or plant or animal viruses.

The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host

organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a ribosome binding site, often along with other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals.

The terms "overexpression" and "overexpressing" and grammatical equivalents, are used in reference to levels of mRNA to indicate a level of expression approximately 3-fold higher (or greater) than that observed in a given tissue in a control or non-transgenic animal. Levels of mRNA are measured using any of a number of techniques known to those skilled in the art including, but not limited to Northern blot analysis. Appropriate controls are included on the Northern blot to control for differences in the amount of RNA loaded from each tissue analyzed (*e.g.*, the amount of 28S rRNA, an abundant RNA transcript present at essentially the same amount in all tissues, present in each sample can be used as a means of normalizing or standardizing the mRNA-specific signal observed on Northern blots). The amount of mRNA present in the band corresponding in size to the correctly spliced transgene RNA is quantified; other minor species of RNA which hybridize to the transgene probe are not considered in the quantification of the expression of the transgenic mRNA.

The term "transfection" as used herein refers to the introduction of foreign DNA into eukaryotic cells. Transfection may be accomplished by a variety of means known to the art including calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection, polybrene-mediated transfection, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, retroviral infection, and biolistics.

The term "calcium phosphate co-precipitation" refers to a technique for the introduction of nucleic acids into a cell. The uptake of nucleic acids by cells is enhanced when the nucleic acid is presented as a calcium phosphate-nucleic acid co-precipitate. The original technique of Graham and van der Eb (Graham and van der Eb, Virol., 52:456 [1973]), has been modified by several groups to optimize conditions for particular types of cells. The art is well aware of these numerous modifications.

The term "stable transfection" or "stably transfected" refers to the introduction and integration of foreign DNA into the genome of the transfected cell. The term "stable

transfectant" refers to a cell that has stably integrated foreign DNA into the genomic DNA.

The term "transient transfection" or "transiently transfected" refers to the introduction of foreign DNA into a cell where the foreign DNA fails to integrate into the genome of the transfected cell. The foreign DNA persists in the nucleus of the transfected cell for several days. During this time the foreign DNA is subject to the regulatory controls that govern the expression of endogenous genes in the chromosomes. The term "transient transfectant" refers to cells that have taken up foreign DNA but have failed to integrate this DNA.

As used herein, the term "selectable marker" refers to the use of a gene that encodes an enzymatic activity that confers the ability to grow in medium lacking what would otherwise be an essential nutrient (*e.g.* the HIS3 gene in yeast cells); in addition, a selectable marker may confer resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed. Selectable markers may be "dominant"; a dominant selectable marker encodes an enzymatic activity that can be detected in any eukaryotic cell line. Examples of dominant selectable markers include the bacterial aminoglycoside 3' phosphotransferase gene (also referred to as the neo gene) that confers resistance to the drug G418 in mammalian cells, the bacterial hygromycin G phosphotransferase (hyg) gene that confers resistance to the antibiotic hygromycin and the bacterial xanthine-guanine phosphoribosyl transferase gene (also referred to as the gpt gene) that confers the ability to grow in the presence of mycophenolic acid. Other selectable markers are not dominant in that their use must be in conjunction with a cell line that lacks the relevant enzyme activity. Examples of non-dominant selectable markers include the thymidine kinase (tk) gene that is used in conjunction with tk^- cell lines, the CAD gene that is used in conjunction with CAD-deficient cells and the mammalian hypoxanthine-guanine phosphoribosyl transferase (hprt) gene that is used in conjunction with $hprt^-$ cell lines. A review of the use of selectable markers in mammalian cell lines is provided in Sambrook, J. *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989) pp.16.9-16.15.

As used herein, the term "cell culture" refers to any in vitro culture of cells. Included within this term are continuous cell lines (*e.g.*, with an immortal phenotype),

primary cell cultures, transformed cell lines, finite cell lines (*e.g.*, non-transformed cells), and any other cell population maintained in vitro.

As used, the term "eukaryote" refers to organisms distinguishable from "prokaryotes." It is intended that the term encompass all organisms with cells that exhibit the usual characteristics of eukaryotes, such as the presence of a true nucleus bounded by a nuclear membrane, within which lie the chromosomes, the presence of membrane-bound organelles, and other characteristics commonly observed in eukaryotic organisms. Thus, the term includes, but is not limited to such organisms as fungi, protozoa, and animals (*e.g.*, humans).

As used herein, the term "*in vitro*" refers to an artificial environment and to processes or reactions that occur within an artificial environment. In vitro environments can consist of, but are not limited to, test tubes and cell culture. The term "*in vivo*" refers to the natural environment (*e.g.*, an animal or a cell) and to processes or reaction that occur within a natural environment.

The terms "test compound" and "candidate compound" refer to any chemical entity, pharmaceutical, drug, and the like that is a candidate for use to treat or prevent a disease, illness, sickness, or disorder of bodily function (*e.g.*, cancer). Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by screening using the screening methods of the present invention. In some embodiments of the present invention, test compounds include antisense compounds.

As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum and the like. Environmental samples include environmental material such as surface matter, soil, water, crystals and industrial samples. Such examples are not however to be construed as limiting the sample types applicable to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compositions and methods for cancer diagnostics, including but not limited to, cancer markers. In particular, the present invention provides gene expression profiles associated with prostate cancers. Accordingly, the present invention provides method of characterizing prostate tissues, kits for the detection of markers, as well as drug screening and therapeutic applications.

I. Markers for Prostate Cancer

The present invention provides markers whose expression is specifically altered in cancerous prostate tissues. Such markers find use in the diagnosis and characterization of prostate cancer.

A. Identification of Markers

Experiments conducted during the development of the present invention resulted in the identification of genes whose expression level was altered (*e.g.*, increased or decreased) in PCA. The methods utilized glass slide cDNA microarrays that included approximately 5000 known, named genes, 4400 ESTs, and 500 control elements, as well as normal and cancerous prostate tissue. Differentially expressed genes were divided into functional clusters. The expression of relevant genes was confirmed using Western blot analysis. Protein expression in prostate tissues was measured for several genes of interest.

The methods of the present invention (*See e.g.*, Example 2) were used to identify clusters of genes that were up or down regulated in PCA, benign prostate tissue, pre-cancerous tissue, and normal prostate. From these clusters, two genes, hepsin and pim-1 were identified as genes that were of particular relevance. Immunohistochemistry (*See e.g.*, Example 4) was used to characterize the presence of hepsin and pim-1 proteins in prostate tissue. Hepsin was found to stain strongly in pre-cancerous tissue (HG-PIN). In addition, hepsin was found to stain less strongly in PCA tissues of men found to have an increased risk of metastasis as measured by PSA failure (increased PSA following surgery), thus confirming the diagnostic utility of hepsin. In addition, deceased expression of pim-1 in PCA tissue was also found to be associated with increased risk of

PSA failure. Accordingly, in some embodiments, the present invention provides methods of detecting and characterizing prostate tissues.

The methods of the present invention identified a further gene alpha-methyl-CoA racemase (AMACR) that was found to be expressed in PCA, but not benign prostate tissue (*See e.g.*, Example 5). AMACR was found to be present in the serum and urine of prostate or bladder cancer patients. In addition, a humoral response to AMACR was identified. In still further embodiments, the methods of the present invention were used to characterize the EZH2 gene. EZH2 was found to be up-regulated in metastatic prostate cancer. The inhibition of EZH2 expression in prostate cells inhibited cell proliferation in vitro, as well as inducing transcriptional repression of a variety of genes. The methods of the present invention further identified CtBP1 and CTBP2, as well as that GP73 as being over-expressed in metastatic prostate cancer relative to localized prostate cancer and benign tissue.

In still further embodiments, the methods of the present invention identified annexins 1, 2, 4, 7 and 11 as being significantly decreased in hormone refractory PCA when compared to localized hormone naïve Pca. Tissue microarray analysis revealed a significant decrease in protein expression for annexins 1, 2, 4, 7 and 11 in hormone refractory PCA as compared to localized Pca. No significant differences were detected between the clinically localized PCA and non-cancerous prostate tissues.

B. Detection of Markers

In some embodiments, the present invention provides methods for detection of expression of cancer markers (*e.g.*, prostate cancer markers). In preferred embodiments, expression is measured directly (*e.g.*, at the RNA or protein level). In some embodiments, expression is detected in tissue samples (*e.g.*, biopsy tissue). In other embodiments, expression is detected in bodily fluids (*e.g.*, including but not limited to, plasma, serum, whole blood, mucus, and urine). The present invention further provides panels and kits for the detection of markers. In preferred embodiments, the presence of a cancer marker is used to provide a prognosis to a subject. For example, the detection of hepsin or pim-1 in prostate tissues is indicative of a cancer that is likely to metastasize and the expression of hepsin is indicative of a pre-cancerous tissue that is likely to

become cancerous. In addition, the expression of AMACR is indicative of cancerous tissue. The information provided is also used to direct the course of treatment. For example, if a subject is found to have a marker indicative of a highly metastasizing tumor, additional therapies (*e.g.*, hormonal or radiation therapies) can be started at a earlier point when they are more likely to be effective (*e.g.*, before metastasis). In addition, if a subject is found to have a tumor that is not responsive to hormonal therapy, the expense and inconvenience of such therapies can be avoided.

The present invention is not limited to the markers described above. Any suitable marker that correlates with cancer or the progression of cancer may be utilized, including but not limited to, those described in the illustrative examples below (*e.g.*, FKBP5, FASN, FOLH1, TNFSF10, PCM1, S100A11, IGFBP3, SLUG, GSTM3, ATF2, RAB5A, IL1R2, ITGB4, CCND2, EDNRB, APP, THROMBOSPONDIN 1, ANNEXIN A1, EPHA1, NCK1, MAPK6, SGK, HEVIN, MEIS2, MYLK, FZD7, CAVEOLIN 2, TACC1, ARHB, PSG9, GSTM1, KERATIN 5, TIMP2, GELSOLIN, ITM2C, GSTM5, VINCULIN, FHL1, GSTP1, MEIS1, ETS2, PPP2CB, CATHEPSIN B, CATHEPSIN H, COL1A2, RIG, VIMENTIN, MOESIN, MCAM, FIBRONECTIN 1, NBL1, ANNEXIN A4, ANEXIN A11, IL1R1, IGFBP5, CYSTATIN C, COL15A1, ADAMTS1, SKI, EGR1, FOSB, CFLAR, JUN, YWHAB, NRAS, C7, SCYA2, ITGA1, LUMICAN, C1S, C4BPA, COL3A1, FAT, MMECD10, CLUSTERIN, PLA2G2A, MADh4, SEPP1, RAB2, PP1CB, MPDZ, PRKCL2, CTBP1, CTBP2, MAP3K10, TBXA2F, MTA1, RAP2, TRAP1, TFCP2, E2EPF, UBCH10, TASTIN, EZH2, FLS353, MYBL2, LIMK1, GP73, VAV2, TOP2A, ASNS, CTBP, AMACR, ABCC5 (MDR5), and TRAF4. Additional markers are also contemplated to be within the scope of the present invention. Any suitable method may be utilized to identify and characterize cancer markers suitable for use in the methods of the present invention, including but not limited to, those described in illustrative Examples 1-15 below. For example, in some embodiments, markers identified as being up or down-regulated in PCA using the gene expression microarray methods of the present invention are further characterized using tissue microarray, immunohistochemistry, Northern blot analysis, siRNA or antisense RNA inhibition, mutation analysis, investigation of expression with clinical outcome, as well as other methods disclosed herein.

In some embodiments, the present invention provides a panel for the analysis of a plurality of markers. The panel allows for the simultaneous analysis of multiple markers correlating with carcinogenesis and/or metastasis. For example, a panel may include markers identified as correlating with cancerous tissue, metastatic cancer, localized cancer that is likely to metastasize, pre-cancerous tissue that is likely to become cancerous, and pre-cancerous tissue that is not likely to become cancerous. Depending on the subject, panels may be analyzed alone or in combination in order to provide the best possible diagnosis and prognosis. Markers for inclusion on a panel are selected by screening for their predictive value using any suitable method, including but not limited to, those described in the illustrative examples below.

In other embodiments, the present invention provides an expression profile map comprising expression profiles of cancers of various stages or prognoses (*e.g.*, likelihood of future metastasis). Such maps can be used for comparison with patient samples. In some embodiments comparisons are made using the method described in Example 2. However, the present invention is not limited to the method described in Example 2. Any suitable method may be utilized, including but not limited to, by computer comparison of digitized data. The comparison data is used to provide diagnoses and/or prognoses to patients.

1. Detection of RNA

In some preferred embodiments, detection of prostate cancer markers (*e.g.*, including but not limited to, those disclosed herein) is detected by measuring the expression of corresponding mRNA in a tissue sample (*e.g.*, prostate tissue). mRNA expression may be measured by any suitable method, including but not limited to, those disclosed below.

In some embodiments, RNA is detection by Northern blot analysis. Northern blot analysis involves the separation of RNA and hybridization of a complementary labeled probe. An exemplary method for Northern blot analysis is provided in Example 3.

In other embodiments, RNA expression is detected by enzymatic cleavage of specific structures (INVADER assay, Third Wave Technologies; *See e.g.*, U.S. Patent Nos. 5,846,717, 6,090,543; 6,001,567; 5,985,557; and 5,994,069; each of which is herein

incorporated by reference). The INVADER assay detects specific nucleic acid (*e.g.*, RNA) sequences by using structure-specific enzymes to cleave a complex formed by the hybridization of overlapping oligonucleotide probes.

In still further embodiments, RNA (or corresponding cDNA) is detected by hybridization to a oligonucleotide probe). A variety of hybridization assays using a variety of technologies for hybridization and detection are available. For example, in some embodiments, TaqMan assay (PE Biosystems, Foster City, CA; *See e.g.*, U.S. Patent Nos. 5,962,233 and 5,538,848, each of which is herein incorporated by reference) is utilized. The assay is performed during a PCR reaction. The TaqMan assay exploits the 5'-3' exonuclease activity of the AMPLITAQ GOLD DNA polymerase. A probe consisting of an oligonucleotide with a 5'-reporter dye (*e.g.*, a fluorescent dye) and a 3'-quencher dye is included in the PCR reaction. During PCR, if the probe is bound to its target, the 5'-3' nucleolytic activity of the AMPLITAQ GOLD polymerase cleaves the probe between the reporter and the quencher dye. The separation of the reporter dye from the quencher dye results in an increase of fluorescence. The signal accumulates with each cycle of PCR and can be monitored with a fluorimeter.

In yet other embodiments, reverse-transcriptase PCR (RT-PCR) is used to detect the expression of RNA. In RT-PCR, RNA is enzymatically converted to complementary DNA or "cDNA" using a reverse transcriptase enzyme. The cDNA is then used as a template for a PCR reaction. PCR products can be detected by any suitable method, including but not limited to, gel electrophoresis and staining with a DNA specific stain or hybridization to a labeled probe. In some embodiments, the quantitative reverse transcriptase PCR with standardized mixtures of competitive templates method described in U.S. Patents 5,639,606, 5,643,765, and 5,876,978 (each of which is herein incorporated by reference) is utilized.

2. Detection of Protein

In other embodiments, gene expression of cancer markers is detected by measuring the expression of the corresponding protein or polypeptide. Protein expression may be detected by any suitable method. In some embodiments, proteins are detected by the immunohistochemistry method of Example 4. In other embodiments, proteins are

detected by their binding to an antibody raised against the protein. The generation of antibodies is described below.

Antibody binding is detected by techniques known in the art (*e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many methods are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In some embodiments, an automated detection assay is utilized. Methods for the automation of immunoassays include those described in U.S. Patents 5,885,530, 4,981,785, 6,159,750, and 5,358,691, each of which is herein incorporated by reference. In some embodiments, the analysis and presentation of results is also automated. For example, in some embodiments, software that generates a prognosis based on the presence or absence of a series of proteins corresponding to cancer markers is utilized.

In other embodiments, the immunoassay described in U.S. Patents 5,599,677 and 5,672,480; each of which is herein incorporated by reference.

3. Data Analysis

In some embodiments, a computer-based analysis program is used to translate the raw data generated by the detection assay (*e.g.*, the presence, absence, or amount of a given marker or markers) into data of predictive value for a clinician. The clinician can access the predictive data using any suitable means. Thus, in some preferred embodiments, the present invention provides the further benefit that the clinician, who is not likely to be trained in genetics or molecular biology, need not understand the raw

data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject.

The present invention contemplates any method capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information provides, medical personal, and subjects. For example, in some embodiments of the present invention, a sample (*e.g.*, a biopsy or a serum or urine sample) is obtained from a subject and submitted to a profiling service (*e.g.*, clinical lab at a medical facility, genomic profiling business, etc.), located in any part of the world (*e.g.*, in a country different than the country where the subject resides or where the information is ultimately used) to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves (*e.g.*, a urine sample) and directly send it to a profiling center. Where the sample comprises previously determined biological information, the information may be directly sent to the profiling service by the subject (*e.g.*, an information card containing the information may be scanned by a computer and the data transmitted to a computer of the profiling center using an electronic communication systems). Once received by the profiling service, the sample is processed and a profile is produced (*i.e.*, expression data), specific for the diagnostic or prognostic information desired for the subject.

The profile data is then prepared in a format suitable for interpretation by a treating clinician. For example, rather than providing raw expression data, the prepared format may represent a diagnosis or risk assessment (e.g., likelihood of metastasis or PSA failure) for the subject, along with recommendations for particular treatment options. The data may be displayed to the clinician by any suitable method. For example, in some embodiments, the profiling service generates a report that can be printed for the clinician (*e.g.*, at the point of care) or displayed to the clinician on a computer monitor.

In some embodiments, the information is first analyzed at the point of care or at a regional facility. The raw data is then sent to a central processing facility for further analysis and/or to convert the raw data to information useful for a clinician or patient. The central processing facility provides the advantage of privacy (all data is stored in a

central facility with uniform security protocols), speed, and uniformity of data analysis. The central processing facility can then control the fate of the data following treatment of the subject. For example, using an electronic communication system, the central facility can provide data to the clinician, the subject, or researchers.

In some embodiments, the subject is able to directly access the data using the electronic communication system. The subject may chose further intervention or counseling based on the results. In some embodiments, the data is used for research use. For example, the data may be used to further optimize the inclusion or elimination of markers as useful indicators of a particular condition or stage of disease.

4. Kits

In yet other embodiments, the present invention provides kits for the detection and characterization of prostate cancer. In some embodiments, the kits contain antibodies specific for a cancer marker, in addition to detection reagents and buffers. In other embodiments, the kits contain reagents specific for the detection of mRNA or cDNA (*e.g.*, oligonucleotide probes or primers). In preferred embodiments, the kits contain all of the components necessary to perform a detection assay, including all controls, directions for performing assays, and any necessary software for analysis and presentation of results.

5. In vivo Imaging

In some embodiments, in vivo imaging techniques are used to visualize the expression of cancer markers in an animal (*e.g.*, a human or non-human mammal). For example, in some embodiments, cancer marker mRNA or protein is labeled using an labeled antibody specific for the cancer marker. A specifically bound and labeled antibody can be detected in an individual using an *in vivo* imaging method, including, but not limited to, radionuclide imaging, positron emission tomography, computerized axial tomography, X-ray or magnetic resonance imaging method, fluorescence detection, and chemiluminescent detection. Methods for generating antibodies to the cancer markers of the present invention are described below.

The in vivo imaging methods of the present invention are useful in the diagnosis of cancers that express the cancer markers of the present invention (*e.g.*, prostate cancer). In vivo imaging is used to visualize the presence of a marker indicative of the cancer. Such techniques allow for diagnosis without the use of an unpleasant biopsy. The in vivo imaging methods of the present invention are also useful for providing prognoses to cancer patients. For example, the presence of a marker indicative of cancers likely to metastasize can be detected. The in vivo imaging methods of the present invention can further be used to detect metastatic cancers in other parts of the body.

In some embodiments, reagents (*e.g.*, antibodies) specific for the cancer markers of the present invention are fluorescently labeled. The labeled antibodies are introduced into a subject (*e.g.*, orally or parenterally). Fluorescently labeled antibodies are detected using any suitable method (*e.g.*, using the apparatus described in U.S. Patent 6,198,107, herein incorporated by reference).

In other embodiments, antibodies are radioactively labeled. The use of antibodies for in vivo diagnosis is well known in the art. Sumerdon *et al.*, (Nucl. Med. Biol 17:247-254 [1990] have described an optimized antibody-chelator for the radioimmunoscintographic imaging of tumors using Indium-111 as the label. Griffin *et al.*, (J Clin Onc 9:631-640 [1991]) have described the use of this agent in detecting tumors in patients suspected of having recurrent colorectal cancer. The use of similar agents with paramagnetic ions as labels for magnetic resonance imaging is known in the art (Lauffer, Magnetic Resonance in Medicine 22:339-342 [1991]). The label used will depend on the imaging modality chosen. Radioactive labels such as Indium-111, Technetium-99m, or Iodine-131 can be used for planar scans or single photon emission computed tomography (SPECT). Positron emitting labels such as Fluorine-19 can also be used for positron emission tomography (PET). For MRI, paramagnetic ions such as Gadolinium (III) or Manganese (II) can be used.

Radioactive metals with half-lives ranging from 1 hour to 3.5 days are available for conjugation to antibodies, such as scandium-47 (3.5 days) gallium-67 (2.8 days), gallium-68 (68 minutes), technetiium-99m (6 hours), and indium-111 (3.2 days), of which gallium-67, technetium-99m, and indium-111 are preferable for gamma camera imaging, gallium-68 is preferable for positron emission tomography.

A useful method of labeling antibodies with such radiometals is by means of a bifunctional chelating agent, such as diethylenetriaminepentaacetic acid (DTPA), as described, for example, by Khaw *et al.* (Science 209:295 [1980]) for In-111 and Tc-99m, and by Scheinberg *et al.* (Science 215:1511 [1982]). Other chelating agents may also be used, but the 1-(p-carboxymethoxybenzyl)EDTA and the carboxycarbonic anhydride of DTPA are advantageous because their use permits conjugation without affecting the antibody's immunoreactivity substantially.

Another method for coupling DPTA to proteins is by use of the cyclic anhydride of DTPA, as described by Hnatowich *et al.* (Int. J. Appl. Radiat. Isot. 33:327 [1982]) for labeling of albumin with In-111, but which can be adapted for labeling of antibodies. A suitable method of labeling antibodies with Tc-99m which does not use chelation with DPTA is the pretinning method of Crockford *et al.*, (U.S. Pat. No. 4,323,546, herein incorporated by reference).

A preferred method of labeling immunoglobulins with Tc-99m is that described by Wong *et al.* (Int. J. Appl. Radiat. Isot., 29:251 [1978]) for plasma protein, and recently applied successfully by Wong *et al.* (J. Nucl. Med., 23:229 [1981]) for labeling antibodies.

In the case of the radiometals conjugated to the specific antibody, it is likewise desirable to introduce as high a proportion of the radiolabel as possible into the antibody molecule without destroying its immunospecificity. A further improvement may be achieved by effecting radiolabeling in the presence of the specific cancer marker of the present invention, to insure that the antigen binding site on the antibody will be protected. The antigen is separated after labeling.

In still further embodiments, *in vivo* biophotonic imaging (Xenogen, Almeda, CA) is utilized for in vivo imaging. This real-time *in vivo* imaging utilizes luciferase. The luciferase gene is incorporated into cells, microorganisms, and animals (*e.g.*, as a fusion protein with a cancer marker of the present invention). When active, it leads to a reaction that emits light. A CCD camera and software is used to capture the image and analyze it.

II. Antibodies

The present invention provides isolated antibodies. In preferred embodiments, the present invention provides monoclonal antibodies that specifically bind to an isolated polypeptide comprised of at least five amino acid residues of the cancer markers described herein (*e.g.*, hepsin, pim-1, AMACR, EZH2, CTBP). These antibodies find use in the diagnostic methods described herein.

An antibody against a protein of the present invention may be any monoclonal or polyclonal antibody, as long as it can recognize the protein. Antibodies can be produced by using a protein of the present invention as the antigen according to a conventional antibody or antiserum preparation process.

The present invention contemplates the use of both monoclonal and polyclonal antibodies. Any suitable method may be used to generate the antibodies used in the methods and compositions of the present invention, including but not limited to, those disclosed herein. For example, for preparation of a monoclonal antibody, protein, as such, or together with a suitable carrier or diluent is administered to an animal (*e.g.*, a mammal) under conditions that permit the production of antibodies. For enhancing the antibody production capability, complete or incomplete Freund's adjuvant may be administered. Normally, the protein is administered once every 2 weeks to 6 weeks, in total, about 2 times to about 10 times. Animals suitable for use in such methods include, but are not limited to, primates, rabbits, dogs, guinea pigs, mice, rats, sheep, goats, etc.

For preparing monoclonal antibody-producing cells, an individual animal whose antibody titer has been confirmed (*e.g.*, a mouse) is selected, and 2 days to 5 days after the final immunization, its spleen or lymph node is harvested and antibody-producing cells contained therein are fused with myeloma cells to prepare the desired monoclonal antibody producer hybridoma. Measurement of the antibody titer in antiserum can be carried out, for example, by reacting the labeled protein, as described hereinafter and antiserum and then measuring the activity of the labeling agent bound to the antibody. The cell fusion can be carried out according to known methods, for example, the method described by Koehler and Milstein (Nature 256:495 [1975]). As a fusion promoter, for example, polyethylene glycol (PEG) or Sendai virus (HVJ), preferably PEG is used.

Examples of myeloma cells include NS-1, P3U1, SP2/0, AP-1 and the like. The proportion of the number of antibody producer cells (spleen cells) and the number of myeloma cells to be used is preferably about 1:1 to about 20:1. PEG (preferably PEG 1000-PEG 6000) is preferably added in concentration of about 10% to about 80%. Cell fusion can be carried out efficiently by incubating a mixture of both cells at about 20°C to about 40°C, preferably about 30°C to about 37°C for about 1 minute to 10 minutes.

Various methods may be used for screening for a hybridoma producing the antibody (*e.g.*, against a tumor antigen or autoantibody of the present invention). For example, where a supernatant of the hybridoma is added to a solid phase (*e.g.*, microplate) to which antibody is adsorbed directly or together with a carrier and then an anti-immunoglobulin antibody (if mouse cells are used in cell fusion, anti-mouse immunoglobulin antibody is used) or Protein A labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the protein bound to the solid phase. Alternately, a supernatant of the hybridoma is added to a solid phase to which an anti-immunoglobulin antibody or Protein A is adsorbed and then the protein labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the protein bound to the solid phase.

Selection of the monoclonal antibody can be carried out according to any known method or its modification. Normally, a medium for animal cells to which HAT (hypoxanthine, aminopterin, thymidine) are added is employed. Any selection and growth medium can be employed as long as the hybridoma can grow. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT medium containing 1% to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku) and the like can be used. Normally, the cultivation is carried out at 20°C to 40°C, preferably 37°C for about 5 days to 3 weeks, preferably 1 week to 2 weeks under about 5% CO_2 gas. The antibody titer of the supernatant of a hybridoma culture can be measured according to the same manner as described above with respect to the antibody titer of the anti-protein in the antiserum.

Separation and purification of a monoclonal antibody (*e.g.*, against a cancer marker of the present invention) can be carried out according to the same manner as those of conventional polyclonal antibodies such as separation and purification of

immunoglobulins, for example, salting-out, alcoholic precipitation, isoelectric point precipitation, electrophoresis, adsorption and desorption with ion exchangers (*e.g.*, DEAE), ultracentrifugation, gel filtration, or a specific purification method wherein only an antibody is collected with an active adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding to obtain the antibody.

Polyclonal antibodies may be prepared by any known method or modifications of these methods including obtaining antibodies from patients. For example, a complex of an immunogen (an antigen against the protein) and a carrier protein is prepared and an animal is immunized by the complex according to the same manner as that described with respect to the above monoclonal antibody preparation. A material containing the antibody against is recovered from the immunized animal and the antibody is separated and purified.

As to the complex of the immunogen and the carrier protein to be used for immunization of an animal, any carrier protein and any mixing proportion of the carrier and a hapten can be employed as long as an antibody against the hapten, which is crosslinked on the carrier and used for immunization, is produced efficiently. For example, bovine serum albumin, bovine cycloglobulin, keyhole limpet hemocyanin, etc. may be coupled to an hapten in a weight ratio of about 0.1 part to about 20 parts, preferably, about 1 part to about 5 parts per 1 part of the hapten.

In addition, various condensing agents can be used for coupling of a hapten and a carrier. For example, glutaraldehyde, carbodiimide, maleimide activated ester, activated ester reagents containing thiol group or dithiopyridyl group, and the like find use with the present invention. The condensation product as such or together with a suitable carrier or diluent is administered to a site of an animal that permits the antibody production. For enhancing the antibody production capability, complete or incomplete Freund's adjuvant may be administered. Normally, the protein is administered once every 2 weeks to 6 weeks, in total, about 3 times to about 10 times.

The polyclonal antibody is recovered from blood, ascites and the like, of an animal immunized by the above method. The antibody titer in the antiserum can be measured according to the same manner as that described above with respect to the supernatant of the hybridoma culture. Separation and purification of the antibody can be

carried out according to the same separation and purification method of immunoglobulin as that described with respect to the above monoclonal antibody.

The protein used herein as the immunogen is not limited to any particular type of immunogen. For example, a cancer marker of the present invention (further including a gene having a nucleotide sequence partly altered) can be used as the immunogen. Further, fragments of the protein may be used. Fragments may be obtained by any methods including, but not limited to expressing a fragment of the gene, enzymatic processing of the protein, chemical synthesis, and the like.

III. Drug Screening

In some embodiments, the present invention provides drug screening assays (*e.g.*, to screen for anticancer drugs). The screening methods of the present invention utilize cancer markers identified using the methods of the present invention (*e.g.*, including but not limited to, hepsin, pim-1, AMACR, EZH2, and CTBP). For example, in some embodiments, the present invention provides methods of screening for compound that alter (*e.g.*, increase or decrease) the expression of cancer marker genes. In some embodiments, candidate compounds are antisense agents (*e.g.*, oligonucleotides) directed against cancer markers. See Section IV below for a discussion of antisense therapy. In other embodiments, candidate compounds are antibodies that specifically bind to a cancer marker of the present invention.

In one screening method, candidate compounds are evaluated for their ability to alter cancer marker expression by contacting a compound with a cell expressing a cancer marker and then assaying for the effect of the candidate compounds on expression. In some embodiments, the effect of candidate compounds on expression of a cancer marker gene is assayed for by detecting the level of cancer marker mRNA expressed by the cell. mRNA expression can be detected by any suitable method. In other embodiments, the effect of candidate compounds on expression of cancer marker genes is assayed by measuring the level of polypeptide encoded by the cancer markers. The level of polypeptide expressed can be measured using any suitable method, including but not limited to, those disclosed herein.

Specifically, the present invention provides screening methods for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to cancer markers of the present invention, have an inhibitory (or stimulatory) effect on, for example, cancer marker expression or cancer markers activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a cancer marker substrate. Compounds thus identified can be used to modulate the activity of target gene products (*e.g.*, cancer marker genes) either directly or indirectly in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions. Compounds which inhibit the activity or expression of cancer markers are useful in the treatment of proliferative disorders, *e.g.*, cancer, particularly metastatic (*e.g.*, androgen independent) prostate cancer.

In one embodiment, the invention provides assays for screening candidate or test compounds that are substrates of a cancer markers protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds that bind to or modulate the activity of a cancer marker protein or polypeptide or a biologically active portion thereof.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone, which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, *e.g.*, Zuckennann *et al.*, J. Med. Chem. 37: 2678-85 [1994]); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are preferred for use with peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) Anticancer Drug Des. 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.*, Proc. Natl. Acad. Sci. U.S.A. 90:6909 [1993]; Erb *et*

al., Proc. Nad. Acad. Sci. USA 91:11422 [1994]; Zuckermann *et al.*, J. Med. Chem. 37:2678 [1994]; Cho *et al.*, Science 261:1303 [1993]; Carrell *et al.*, Angew. Chem. Int. Ed. Engl. 33.2059 [1994]; Carell *et al.*, Angew. Chem. Int. Ed. Engl. 33:2061 [1994]; and Gallop *et al.*, J. Med. Chem. 37:1233 [1994].

Libraries of compounds may be presented in solution (*e.g.*, Houghten, Biotechniques 13:412-421 [1992]), or on beads (Lam, Nature 354:82-84 [1991]), chips (Fodor, Nature 364:555-556 [1993]), bacteria or spores (U.S. Patent No. 5,223,409; herein incorporated by reference), plasmids (Cull *et al.*, Proc. Nad. Acad. Sci. USA 89:18651869 [1992]) or on phage (Scott and Smith, Science 249:386-390 [1990]; Devlin Science 249:404-406 [1990]; Cwirla *et al.*, Proc. NatI. Acad. Sci. 87:6378-6382 [1990]; Felici, J. Mol. Biol. 222:301 [1991]).

In one embodiment, an assay is a cell-based assay in which a cell that expresses a cancer marker protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to the modulate cancer marker's activity is determined. Determining the ability of the test compound to modulate cancer marker activity can be accomplished by monitoring, for example, changes in enzymatic activity. The cell, for example, can be of mammalian origin.

The ability of the test compound to modulate cancer marker binding to a compound, *e.g.*, a cancer marker substrate, can also be evaluated. This can be accomplished, for example, by coupling the compound, *e.g.*, the substrate, with a radioisotope or enzymatic label such that binding of the compound, *e.g.*, the substrate, to a cancer marker can be determined by detecting the labeled compound, *e.g.*, substrate, in a complex.

Alternatively, the cancer marker is coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate cancer marker binding to a cancer markers substrate in a complex. For example, compounds (*e.g.*, substrates) can be labeled with ^{125}I, ^{35}S ^{14}C or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

The ability of a compound (*e.g.*, a cancer marker substrate) to interact with a cancer marker with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with a cancer marker without the labeling of either the compound or the cancer marker (McConnell *et al.* Science 257:1906-1912 [1992]). As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and cancer markers.

In yet another embodiment, a cell-free assay is provided in which a cancer marker protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the cancer marker protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the cancer markers proteins to be used in assays of the present invention include fragments that participate in interactions with substrates or other proteins, *e.g.*, fragments with high surface probability scores.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

The interaction between two molecules can also be detected, *e.g.*, using fluorescence energy transfer (FRET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos *et al.*, U.S. Patent No. 4,968,103; each of which is herein incorporated by reference). A fluorophore label is selected such that a first donor molecule's emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy.

Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can

be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in 1 5 the assay should be maximal. An FRET binding event can be conveniently measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

In another embodiment, determining the ability of the cancer markers protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (*see, e.g.*, Sjolander and Urbaniczky, Anal. Chem. 63:2338-2345 [1991] and Szabo *et al.* Curr. Opin. Struct. Biol. 5:699-705 [1995]). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BlAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal that can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize cancer markers, an anti-cancer marker antibody or its target molecule to facilitate separation of complexed from non-complexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a cancer marker protein, or interaction of a cancer marker protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase-cancer marker fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione Sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione-derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or cancer

marker protein, and the mixture incubated under conditions conducive for complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above.

Alternatively, the complexes can be dissociated from the matrix, and the level of cancer markers binding or activity determined using standard techniques. Other techniques for immobilizing either cancer markers protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated cancer marker protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, EL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-IgG antibody).

This assay is performed utilizing antibodies reactive with cancer marker protein or target molecules but which do not interfere with binding of the cancer markers protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or cancer markers protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the cancer marker protein or target molecule, as well as enzyme-linked

assays which rely on detecting an enzymatic activity associated with the cancer marker protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including, but not limited to: differential centrifugation (see, for example, Rivas and Minton, Trends Biochem Sci 18:284-7 [1993]); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (*see, e.g.*, Ausubel *et al.*, eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York.); and immunoprecipitation (*see*, for example, Ausubel *et al.*, eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (*See e.g.*, Heegaard J. Mol. Recognit 11:141-8 [1998]; Hageand Tweed J. Chromatogr. Biomed. Sci. Appl 699:499-525 [1997]). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

The assay can include contacting the cancer markers protein or biologically active portion thereof with a known compound that binds the cancer marker to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a cancer marker protein, wherein determining the ability of the test compound to interact with a cancer marker protein includes determining the ability of the test compound to preferentially bind to cancer markers or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

To the extent that cancer markers can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins, inhibitors of such an interaction are useful. A homogeneous assay can be used can be used to identify inhibitors.

For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared such that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent No. 4,109,496, herein incorporated by reference, that utilizes this approach for immunoassays). The addition of

a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified. Alternatively, cancer markers protein can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.*, Cell 72:223-232 [1993]; Madura *et al.*, J. Biol. Chem. 268.12046-12054 [1993]; Bartel *et al.*, Biotechniques 14:920-924 [1993]; Iwabuchi *et al.*, Oncogene 8:1693-1696 [1993]; and Brent W0 94/10300; each of which is herein incorporated by reference), to identify other proteins, that bind to or interact with cancer markers ("cancer marker-binding proteins" or "cancer marker-bp") and are involved in cancer marker activity. Such cancer marker-bps can be activators or inhibitors of signals by the cancer marker proteins or targets as, for example, downstream elements of a cancer markers-mediated signaling pathway.

Modulators of cancer markers expression can also be identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of cancer marker mRNA or protein evaluated relative to the level of expression of cancer marker mRNA or protein in the absence of the candidate compound. When expression of cancer marker mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of cancer marker mRNA or protein expression. Alternatively, when expression of cancer marker mRNA or protein is less (i.e., statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of cancer marker mRNA or protein expression. The level of cancer markers mRNA or protein expression can be determined by methods described herein for detecting cancer markers mRNA or protein.

A modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a cancer markers protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for a disease (*e.g.*, an animal with prostate cancer or metastatic prostate cancer; or an animal harboring a xenograft of a prostate cancer from an animal (*e.g.*, human) or cells from a cancer resulting from metastasis of a prostate cancer (*e.g.*, to a lymph node, bone, or liver), or cells from a prostate cancer cell line.

This invention further pertains to novel agents identified by the above-described screening assays (*See e.g.*, below description of cancer therapies). Accordingly, it is within the scope of this invention to further use an agent identified as described herein (*e.g.*, a cancer marker modulating agent, an antisense cancer marker nucleic acid molecule, a siRNA molecule, a cancer marker specific antibody, or a cancer marker-binding partner) in an appropriate animal model (such as those described herein) to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be, *e.g.*, used for treatments as described herein.

IV. Cancer Therapies

In some embodiments, the present invention provides therapies for cancer (*e.g.*, prostate cancer). In some embodiments, therapies target cancer markers (*e.g.*, including but not limited to, hepsin, pim-1, AMACR, EZH2, and CTBP).

A. Antisense Therapies

In some embodiments, the present invention targets the expression of cancer markers. For example, in some embodiments, the present invention employs compositions comprising oligomeric antisense compounds, particularly oligonucleotides (*e.g.*, those identified in the drug screening methods described above), for use in modulating the function of nucleic acid molecules encoding cancer markers of the present invention, ultimately modulating the amount of cancer marker expressed. This is accomplished by providing antisense compounds that specifically hybridize with one or more nucleic acids encoding cancer markers of the present invention. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds that specifically hybridize to it is generally referred to as "antisense." The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity that

may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of cancer markers of the present invention. In the context of the present invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. For example, expression may be inhibited to potentially prevent tumor proliferation.

It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of the present invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is a nucleic acid molecule encoding a cancer marker of the present invention. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, *e.g.*, detection or modulation of expression of the protein, will result. Within the context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). Eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the present invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA molecule transcribed from a gene encoding a tumor antigen of the present invention, regardless of the sequence(s) of such codons.

Translation termination codon (or "stop codon") of a gene may have one of three sequences (*i.e.*, 5'-UAA, 5'-UAG and 5'-UGA; the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (*i.e.*, 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (*i.e.*, 5' or 3') from a translation termination codon.

The open reading frame (ORF) or "coding region," which refers to the region between the translation initiation codon and the translation termination codon, is also a region that may be targeted effectively. Other target regions include the 5' untranslated region (5' UTR), referring to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3' UTR), referring to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The cap region may also be a preferred target region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," that are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA splice sites (*i.e.*, intron-exon junctions) may also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that

introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

In some embodiments, target sites for antisense inhibition are identified using commercially available software programs (e.g., Biognostik, Gottingen, Germany; SysArris Software, Bangalore, India; Antisense Research Group, University of Liverpool, Liverpool, England; GeneTrove, Carlsbad, CA). In other embodiments, target sites for antisense inhibition are identified using the accessible site method described in U.S. Patent WO0198537A2, herein incorporated by reference.

Once one or more target sites have been identified, oligonucleotides are chosen that are sufficiently complementary to the target (*i.e.*, hybridize sufficiently well and with sufficient specificity) to give the desired effect. For example, in preferred embodiments of the present invention, antisense oligonucleotides are targeted to or near the start codon.

In the context of this invention, "hybridization," with respect to antisense compositions and methods, means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds. It is understood that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired (*i.e.*, under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and in the case of *in vitro* assays, under conditions in which the assays are performed).

Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with specificity, can be used to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway.

The specificity and sensitivity of antisense is also applied for therapeutic uses. For example, antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides are useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues, and animals, especially humans.

While antisense oligonucleotides are a preferred form of antisense compound, the present invention comprehends other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics such as are described below. The antisense compounds in accordance with this invention preferably comprise from about 8 to about 30 nucleobases (*i.e.*, from about 8 to about 30 linked bases), although both longer and shorter sequences may find use with the present invention. Particularly preferred antisense compounds are antisense oligonucleotides, even more preferably those comprising from about 12 to about 25 nucleobases.

Specific examples of preferred antisense compounds useful with the present invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage (*i.e.*, the backbone) of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen *et al.*, Science 254:1497 (1991).

Most preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2, --NH--O--CH_2--, --CH_2--N(CH_3)--O--CH_2-- [known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2--, and --O--N(CH_3)--CH_2--CH_2-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No.

5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, Helv. Chim. Acta 78:486 [1995]) *i.e.*, an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy (*i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group), also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$.

Other preferred modifications include 2'-methoxy(2'-O--CH_3), 2'-aminopropoxy(2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2. degree °C and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Another modification of the oligonucleotides of the present invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, (*e.g.*, hexyl-S-tritylthiol), a thiocholesterol, an aliphatic chain, (*e.g.*, dodecandiol or undecyl residues), a phospholipid, (*e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate), a polyamine or a polyethylene glycol chain or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

One skilled in the relevant art knows well how to generate oligonucleotides containing the above-described modifications. The present invention is not limited to the antisensce oligonucleotides described above. Any suitable modification or substitution may be utilized.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds that are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of the present invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNaseH is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

Chimeric antisense compounds of the present invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above.

The present invention also includes pharmaceutical compositions and formulations that include the antisense compounds of the present invention as described below.

B. Genetic Therapies

The present invention contemplates the use of any genetic manipulation for use in modulating the expression of cancer markers of the present invention. Examples of genetic manipulation include, but are not limited to, gene knockout (*e.g.*, removing the cancer marker gene from the chromosome using, for example, recombination), expression of antisense constructs with or without inducible promoters, and the like. Delivery of nucleic acid construct to cells *in vitro* or *in vivo* may be conducted using any suitable method. A suitable method is one that introduces the nucleic acid construct into the cell such that the desired event occurs (*e.g.*, expression of an antisense construct).

Introduction of molecules carrying genetic information into cells is achieved by any of various methods including, but not limited to, directed injection of naked DNA constructs, bombardment with gold particles loaded with said constructs, and macromolecule mediated gene transfer using, for example, liposomes, biopolymers, and the like. Preferred methods use gene delivery vehicles derived from viruses, including, but not limited to, adenoviruses, retroviruses, vaccinia viruses, and adeno-associated viruses. Because of the higher efficiency as compared to retroviruses, vectors derived from adenoviruses are the preferred gene delivery vehicles for transferring nucleic acid molecules into host cells *in vivo*. Adenoviral vectors have been shown to provide very efficient *in vivo* gene transfer into a variety of solid tumors in animal models and into human solid tumor xenografts in immune-deficient mice. Examples of adenoviral vectors and methods for gene transfer are described in PCT publications WO 00/12738 and WO 00/09675 and U.S. Pat. Appl. Nos. 6,033,908, 6,019,978, 6,001,557, 5,994,132, 5,994,128, 5,994,106, 5,981,225, 5,885,808, 5,872,154, 5,830,730, and 5,824,544, each of which is herein incorporated by reference in its entirety.

Vectors may be administered to subject in a variety of ways. For example, in some embodiments of the present invention, vectors are administered into tumors or tissue associated with tumors using direct injection. In other embodiments, administration is via the blood or lymphatic circulation (*See e.g.*, PCT publication 99/02685 herein incorporated by reference in its entirety). Exemplary dose levels of adenoviral vector are preferably 10^8 to 10^{11} vector particles added to the perfusate.

C. Antibody Therapy

In some embodiments, the present invention provides antibodies that target prostate tumors that express a cancer marker of the present invention (*e.g.*, hepsin, pim-1, EZH2, Annexin, CTBP, GP73, and AMACR). Any suitable antibody (*e.g.*, monoclonal, polyclonal, or synthetic) may be utilized in the therapeutic methods disclosed herein. In preferred embodiments, the antibodies used for cancer therapy are humanized antibodies. Methods for humanizing antibodies are well known in the art (*See e.g.*, U.S. Patents 6,180,370, 5,585,089, 6,054,297, and 5,565,332; each of which is herein incorporated by reference).

In some embodiments, the therapeutic antibodies comprise an antibody generated against a cancer marker of the present invention (*e.g.*, hepsin, pim-1, EZH2, Annexin, CTBP, GP73, and AMACR), wherein the antibody is conjugated to a cytotoxic agent. In such embodiments, a tumor specific therapeutic agent is generated that does not target normal cells, thus reducing many of the detrimental side effects of traditional chemotherapy. For certain applications, it is envisioned that the therapeutic agents will be pharmacologic agents that will serve as useful agents for attachment to antibodies, particularly cytotoxic or otherwise anticellular agents having the ability to kill or suppress the growth or cell division of endothelial cells. The present invention contemplates the use of any pharmacologic agent that can be conjugated to an antibody, and delivered in active form. Exemplary anticellular agents include chemotherapeutic agents, radioisotopes, and cytotoxins. The therapeutic antibodies of the present invention may include a variety of cytotoxic moieties, including but not limited to, radioactive isotopes (*e.g.*, iodine-131, iodine-123, technicium-99m, indium-111, rhenium-188, rhenium-186, gallium-67, copper-67, yttrium-90, iodine-125 or astatine-211), hormones such as a steroid, antimetabolites such as cytosines (*e.g.*, arabinoside, fluorouracil, methotrexate or aminopterin; an anthracycline; mitomycin C), vinca alkaloids (*e.g.*, demecolcine; etoposide; mithramycin), and antitumor alkylating agent such as chlorambucil or melphalan. Other embodiments may include agents such as a coagulant, a cytokine, growth factor, bacterial endotoxin or the lipid A moiety of bacterial endotoxin. For example, in some embodiments, therapeutic agents will include plant-, fungus- or bacteria-derived toxin, such as an A chain toxins, a ribosome inactivating protein, α-

sarcin, aspergillin, restrictocin, a ribonuclease, diphtheria toxin or pseudomonas exotoxin, to mention just a few examples. In some preferred embodiments, deglycosylated ricin A chain is utilized.

In any event, it is proposed that agents such as these may, if desired, be successfully conjugated to an antibody, in a manner that will allow their targeting, internalization, release or presentation to blood components at the site of the targeted tumor cells as required using known conjugation technology (*See, e.g.*, Ghose *et al.*, Methods Enzymol., 93:280 [1983]).

For example, in some embodiments the present invention provides immunotoxins targeted a cancer marker of the present invention (*e.g.*, hepsin, pim-1, EZH2, Annexin, CTBP, GP73, and AMACR). Immunotoxins are conjugates of a specific targeting agent typically a tumor-directed antibody or fragment, with a cytotoxic agent, such as a toxin moiety. The targeting agent directs the toxin to, and thereby selectively kills, cells carrying the targeted antigen. In some embodiments, therapeutic antibodies employ crosslinkers that provide high *in vivo* stability (Thorpe *et al.*, Cancer Res., 48:6396 [1988]).

In other embodiments, particularly those involving treatment of solid tumors, antibodies are designed to have a cytotoxic or otherwise anticellular effect against the tumor vasculature, by suppressing the growth or cell division of the vascular endothelial cells. This attack is intended to lead to a tumor-localized vascular collapse, depriving the tumor cells, particularly those tumor cells distal of the vasculature, of oxygen and nutrients, ultimately leading to cell death and tumor necrosis.

In preferred embodiments, antibody based therapeutics are formulated as pharmaceutical compositions as described below. In preferred embodiments, administration of an antibody composition of the present invention results in a measurable decrease in cancer (*e.g.*, decrease or elimination of tumor).

D. Pharmaceutical Compositions

The present invention further provides pharmaceutical compositions (*e.g.*, comprising the antisense or antibody compounds described above). The pharmaceutical compositions of the present invention may be administered in a number of ways

depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary (*e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, *e.g.*, intrathecal or intraventricular, administration.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product.

Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (WO 97/30731), also enhance the cellular uptake of oligonucleotides.

The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more antisense compounds and (b) one or more other chemotherapeutic agents that function by a non-antisense mechanism. Examples of such chemotherapeutic agents include, but are not limited to, anticancer drugs such as daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin and diethylstilbestrol (DES). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. The administering physician can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in *in vitro* and *in vivo* animal models or based on the examples described herein. In general, dosage is from 0.01 µg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the subject undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 µg to 100 g per kg of body weight, once or more daily, to once every 20 years.

V. Transgenic Animals Expressing Cancer Marker Genes

The present invention contemplates the generation of transgenic animals comprising an exogenous cancer marker gene of the present invention or mutants and variants thereof (*e.g.*, truncations or single nucleotide polymorphisms). In preferred embodiments, the transgenic animal displays an altered phenotype (*e.g.*, increased or decreased presence of markers) as compared to wild-type animals. Methods for analyzing the presence or absence of such phenotypes include but are not limited to, those disclosed herein. In some preferred embodiments, the transgenic animals further display an increased or decreased growth of tumors or evidence of cancer.

The transgenic animals of the present invention find use in drug (*e.g.*, cancer therapy) screens. In some embodiments, test compounds (*e.g.*, a drug that is suspected of being useful to treat cancer) and control compounds (*e.g.*, a placebo) are administered to the transgenic animals and the control animals and the effects evaluated.

The transgenic animals can be generated via a variety of methods. In some embodiments, embryonal cells at various developmental stages are used to introduce transgenes for the production of transgenic animals. Different methods are used depending on the stage of development of the embryonal cell. The zygote is the best target for micro-injection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter that allows reproducible injection of 1-2 picoliters (pl) of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host genome before the first cleavage (Brinster *et al.*, Proc. Natl. Acad. Sci. USA 82:4438-4442 [1985]). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. U.S. Patent No. 4,873,191 describes a method for the micro-injection of zygotes; the disclosure of this patent is incorporated herein in its entirety.

In other embodiments, retroviral infection is used to introduce transgenes into a non-human animal. In some embodiments, the retroviral vector is utilized to transfect oocytes by injecting the retroviral vector into the perivitelline space of the oocyte (U.S. Pat. No. 6,080,912, incorporated herein by reference). In other embodiments, the

developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Janenich, Proc. Natl. Acad. Sci. USA 73:1260 [1976]). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan *et al.*, in *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. [1986]). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner *et al.*, Proc. Natl. Acad Sci. USA 82:6927 [1985]). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Stewart, *et al.*, EMBO J., 6:383 [1987]). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner *et al.*, Nature 298:623 [1982]). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of cells that form the transgenic animal. Further, the founder may contain various retroviral insertions of the transgene at different positions in the genome that generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germline, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner *et al., supra* [1982]). Additional means of using retroviruses or retroviral vectors to create transgenic animals known to the art involve the micro-injection of retroviral particles or mitomycin C-treated cells producing retrovirus into the perivitelline space of fertilized eggs or early embryos (PCT International Application WO 90/08832 [1990], and Haskell and Bowen, Mol. Reprod. Dev., 40:386 [1995]).

In other embodiments, the transgene is introduced into embryonic stem cells and the transfected stem cells are utilized to form an embryo. ES cells are obtained by culturing pre-implantation embryos *in vitro* under appropriate conditions (Evans *et al.*, Nature 292:154 [1981]; Bradley *et al.*, Nature 309:255 [1984]; Gossler *et al.*, Proc. Acad. Sci. USA 83:9065 [1986]; and Robertson *et al.*, Nature 322:445 [1986]). Transgenes can be efficiently introduced into the ES cells by DNA transfection by a variety of methods known to the art including calcium phosphate co-precipitation, protoplast or spheroplast fusion, lipofection and DEAE-dextran-mediated transfection. Transgenes may also be introduced into ES cells by retrovirus-mediated transduction or by micro-injection. Such

transfected ES cells can thereafter colonize an embryo following their introduction into the blastocoel of a blastocyst-stage embryo and contribute to the germ line of the resulting chimeric animal (for review, *See*, Jaenisch, Science 240:1468 [1988]). Prior to the introduction of transfected ES cells into the blastocoel, the transfected ES cells may be subjected to various selection protocols to enrich for ES cells which have integrated the transgene assuming that the transgene provides a means for such selection. Alternatively, the polymerase chain reaction may be used to screen for ES cells that have integrated the transgene. This technique obviates the need for growth of the transfected ES cells under appropriate selective conditions prior to transfer into the blastocoel.

In still other embodiments, homologous recombination is utilized to knock-out gene function or create deletion mutants (*e.g.*, truncation mutants). Methods for homologous recombination are described in U.S. Pat. No. 5,614,396, incorporated herein by reference.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: N (normal); M (molar); mM (millimolar); μM (micromolar); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); pmol (picomoles); g (grams); mg (milligrams); μg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); and°C (degrees Centigrade).

Example 1

Preparation of Total RNA and Reference Pools

The prostate surgical specimens were obtained from The University of Michigan Specialized Research Program in Prostate Cancer (S.P.O.R.E.) Tumor Bank with Institutional Review Board approval. Tumors samples were derived from patients with clinically localized and advanced hormone refractory prostate cancer. Table 1 shows the

samples used in the present studies. All patients were operated on between 1993 and 1998 for clinically localized prostate cancer as determined by preoperative PSA, digital-rectal examination, and prostate needle biopsy. In addition, a subset of patients received bone and CAT scans to evaluate the possibility of metastatic spread. All patients received radical prostatectomy as a monotherapy (*i.e.*, no hormonal or radiation therapy). The advanced prostate tumors were collected from a series of 12 rapid autopsies performed at the University of Michigan on men who died of hormone refractory prostate cancer. In brief, the majority of these patients had either widely metastatic prostate cancer which was treated with hormonal therapy followed by chemotherapy, or patients who presented with clinically localized disease which progressed and were then treated with both hormonal and chemotherapy. The majority of cases had multiple metastatic lesions to numerous sites. All autopsies were performed within 4-6 hours after death. The clinical and pathologic findings of these cases have recently been reported (Rubin *et al.*, Clin. Cancer Res., 6:1038 [2000]). All samples used for the tissue microarray study were fixed in 10% formalin.

Tissues were homogenized using a polytron homogenizer (Brinkman Instruments) in Trizol (Gibco BRL) and the total RNA was isolated according to the standard Trizol protocol. The total RNA obtained was further subjected to an additional round of phenol chloroform extraction, precipitated and resuspended in RNAse free water. Total RNA was quantitated by spectrophotmetric (260/280nm) absorbance and integrity judged by denaturing- formaldehyde agarose gel electrophoresis. Total RNA from four normal tissues was combined in equal concentrations to obtain the reference pool. The human prostate total RNA used in the commercial reference pool was obtained from Clontech, Inc.

Table 1

Prostate Samples

ID	PSA level	Tissue	Gleason Score

BPH-201	6.2	Prostate	NA
BPH-202	3.9	Prostate	NA
BPH-203	3.9	Prostate	NA
BPH-204	4.6	Prostate	NA
BPH-205	4.6	Prostate	NA
BPH-206	4.6	Prostate	NA
BPH-207	4.8	Prostate	NA
BPH-208	13.6	Prostate	NA
BPH-209	9.8	Prostate	NA
BPH-210	4.6	Prostate	NA
BPH-211	2.6	Prostate	NA
BPH-212	7.1	Prostate	NA
BPH-214		Prostate	NA
BPH-215	5.4	Prostate	NA
Prostatitis	9.8	Prostate	NA
NAP-101	4.6	Prostate	NA
NAP-102	9.8	Prostate	NA

NAP-104	7	Prostate	NA
NAP-105	0.09	Prostate	NA
NAP-107	4.7	Prostate	NA
PCA-401	5.2	Prostate	4+4
PCA-402	22	Prostate	4+3
PCA-403	4.7	Prostate	3+3
PCA-404	8.5	Prostate	3+3
PCA-405	4.6	Prostate	3+3
PCA-406	7.8	Prostate	3+3
PCA-407	7.8	Prostate	3+3
PCA-408	5.4	Prostate	3+3
PCA-409	7	Prostate	3+3
PCA-410	44.6	Prostate	4+4
PCA-414		Prostate	3+4
PCA-416	24.1	Prostate	4+4
PCA-417	12.4	Prostate	4+4
PCA-420		Prostate	3+3

PCA-421	13.6	Prostate	3+4
MET-301		Lung	NA
MET-302		Liver	NA
MET-303		Liver	NA
MET-304		Stomach	NA
MET-305		Adrenal	NA
MET-306		Prostate	NA
MET-307		Lymph Node	NA
MET-308		Lymph Node	NA
MET-309		Lymph Node	NA
MET-310		Liver	NA
MET-311		Soft tissue	NA
MET-312		Liver	NA
MET-313		Soft tissue	NA
MET-314		Soft tissue	NA
MET-315		Soft tissue	NA
MET-316		Soft tissue	NA

MET-317		Liver	NA
MET-318		bone	NA
MET-319		bone	NA
MET-320		bone	NA

Table 1. Samples employed in the study. Designating PSA level in ng/mL , Organ sources and Gleason scores. Normal adjacent prostate (NAP), Benign prostatic hyperplasia (BPH), Localized prostate cancer (PCA) and Hormone refractory metastatic prostate cancer (MET). NA refers to "not applicable".

Example 2

Microarray Analysis

This example describes the use of microarray analysis to identify genes that demonstrate an altered level of expression in cancerous or benign prostate tissues.

A. Experimental Methods

Microarray analysis of gene expression was conducted essentially as described by the Brown and Derisi Labs (available at the Internet site www.microarrays.org). The sequence-verified cDNA clones on the human cDNA microarray are available from the web site of Research Genetics. Based on the latest Unigene build, the 10K human cDNA microarray used covers approximately 5520 known, named genes and 4464 ESTs. All chips have various control elements that include human, rat, and yeast genomic DNAs, SSC, yeast genes and "housekeeping genes," among others. In addition, 500 cancer- and apoptosis- related cDNAs from Research Genetics were used to serve as independent controls for clone tracking and function as duplicates for quality control. Three metastatic prostate cancer cell lines: DU-145, LnCAP, and PC3 were also profiled for gene expression.

Fluorescently labeled (Cy5) cDNA was prepared from total RNA from each experimental sample. The two reference samples used in this study were labeled using a second distinguishable fluorescent dye (Cy3) and included a pool of normal adjacent prostate tissue (NAP) from four patients (distinct from those used in the experimental samples) and a commercial pool of normal prostate tissues (CP). In addition to minimizing patient-to-patient variation, comparisons against pools of normal prostate tissue facilitate the discovery of genes that molecularly distinguish prostate neoplasms. The two reference pools are different in that one is comprised of normal adjacent prostate tissue, which may be influenced by paracrine effects mediated by PCA, and furthermore is exposed to the same environmental and genetic factors as the adjacent PCA. By contrast, the CP pool is derived from 19 individuals with no known prostate pathology and also represents a renewable commercially available reference resource.

Purified PCR products, generated using the clone inserts as template, were spotted onto poly-L- lysine coated microscope slides using an Omnigrid robotic arrayer (GeneMachines, CA) equipped with quill-type pins (Majer Scientific, AZ). One full print run generated approximately 100 DNA microarrays. Protocols for printing and post-processing of arrays are well known in the art.

B. Data analysis

Primary analysis was done using the Genepix software package. Images of scanned microarrays were gridded and linked to a gene print list. Initially, data was viewed as a scatter plot of Cy3 versus Cy5 intensities. Cy3 to Cy5 ratios were determined for the individual genes along with various other quality control parameters (*e.g.*, intensity over local background). The Genepix software analysis package flags spots as absent based on spot characteristics (refer to the web site of Axon Instruments, Inc.). Bad spots or areas of the array with obvious defects were manually flagged. Spots with small diameters (< 50 microns) and spots with low signal strengths <350 fluorescence intensity units over local background in the more intense channel were discarded. Flagged spots were not included in subsequent analyses. Data were scaled such that the average median ratio value for all spots was normalized to 1.0 per array.

These files were then imported into a Microsoft Access database. The data for the required experiments were extracted from the database in a single table format with each row representing an array element, each column a hybridization and each cell the observed normalized median of ratios for the array element of the appropriate hybridization. Prior to clustering, the normalized median of ratio values of the genes were log base 2 transformed and filtered for presence across arrays and selected for expression levels and patterns depending on the experimental set as stated. Average linkage hierarchial clustering of an uncentered Pearson correlation similarity matrix was applied using the program Cluster (Eisen *et al.*, PNAS 95:14863 [1998]), and the results were analyzed and figures generated with the program TreeView. TreeView and Cluster are available from Michael Eisen's lab at the Lawrence Berkeley National Lab.

C. Results

Over forty 10K human cDNA microarrays were used to assess gene expression in four clinical states of prostate-derived tissues in relation to two distinct reference pools of normal specimens. Figure 1 provides an overview of the variation in gene expression across the different tissue specimens analyzed. A hierarchical clustering algorithm was employed to group genes and experimental samples based on similarities of gene expression patterns over all the genes and samples tested, respectively.

1. Expression Dendrograms

Relationships between the experimental samples are summarized as dendrograms (Figure 1a), in which the pattern and length of the branches reflect the relatedness of the samples. Figure 1a shows dendrograms that reveal the variation in gene expression pattern between experimental samples with the two references employed. Individual samples in each group are indicated by the branches of the same color whereby METs are in dark blue, localized PCAs in orange, NAPs in light blue, BPHs in gray, and cell lines in pink. Asterisk (*) indicates a sample that was initially documented as BPH, but was later confirmed to have 5% cancer tissue. The details of metastatic samples used in this study are as follows: MET 301, from Lung; MET 302 and 303 from liver; MET 304, from stomach; MET 305 from adrenal gland; MET 306 from prostate; and MET 307 was

from lymph node. Hierarchical clustering of the data identified distinct patterns of gene expression between the various groups analyzed. Each row represents a single gene with 1520 genes depicted in b, and 1006 genes depicted in c. The results represent the ratio of hybridization of fluorescent cDNA probes prepared from each experimental mRNA to the respective reference pools. These ratios are a measure of relative gene expression in each experimental sample and are depicted according to the color scale at the bottom left. Red and green colors in the matrix represent genes that are up- and down-regulated, respectively, relative to the reference pool employed. Black lines in the matrix represent transcript levels that are unchanged, while gray lines signify technically inadequate or missing data (NP, not present). Color saturation reflects the magnitude of the ratio relative to the median for each set of samples.

Figure 1b shows a cluster diagram of the various sample groups compared against normal adjacent prostate pool as reference. Data obtained in the expression profiling experiments included CBCR-t Index number, Clone, ID, Unigene Cluster Ids, Accession ID, NID, gene symbol, and name fields for each gene used in the array. The name field contains genes having partial or complete homology based on homology searches. In addition, the data contains the numerical difference in expression levels compared to the reference pool for each gene. Prior to hierarchical average-linkage clustering, the data was filtered for at least a 2–fold change in expression ratio and ratio measurements present in 50% of the samples. By this method, 1520 genes were selected from the NAP reference data set. Indicated by vertical bars on the left (b1 to b6) of Figure 1b are regions identified with characteristic gene expression signatures. Clusters b1 and b5 show genes up-regulated in localized PCA but not in metastatic PCA. Clusters b2 and b4 highlight genes down-regulated in metastatic PCA and the cell lines DU145 and LnCAP. Cluster b3 identifies genes down-regulated in both localized PCA and metastatic PCA. Cluster b6 highlights genes that are primarily up-regulated in metastatic PCA alone. Portions of Clusters b4 and b6 are shown enlarged with selected genes shown using Human genome organization (HUGO) gene nomenclature.

Figure 1c shows a cluster diagram of the various sample groups compared against the commercial prostate pool reference. Prior to hierarchical average-linkage clustering, the data was filtered for at least a 3–fold change in expression ratio and ratio

measurements present in 75% of the samples resulting in a total of 1006 genes. Regions with distinct patterns (c1-c6) are indicated by vertical bars to the right of Figure 1c. Cluster c1 depicts genes down-regulated in both localized PCA and metastatic PCA. Cluster c2 represents genes down-regulated only in metastatic PCA. Cluster c3 shows genes that are highly represented in the commercial pool. Cluster c4 highlights genes that are up-regulated in localized PCA and in metastatic PCA. Cluster c5 represent genes with a low representation in the commercial pool. Cluster c6, represents genes that are down-regulated in metastatic PCA but are up-regulated in all other samples used.

Benign conditions of the prostate such as BPH and NAP cluster separately from malignant PCA cell lines or tissues, regardless of the reference pool used. Within the PCA cluster, it is also evident that metastatic PCA and clinically localized PCA formed distinct subgroups. Similarly, in the "benign" grouping, BPH tended to distinguish itself from NAP. Interestingly, one of the "BPH" samples initially clustered with the localized PCA group. Upon further histopathologic review, however, it was discovered that this sample contained a small focus of neoplastic tissue (~5%), thus accounting for its initial misclassification (now designated PCA+BPH in Figure 1a).

Eisen matrix formats (Eisen *et al., supra*) of the variation in gene expression are also presented (Figure 1 b and 1c). With a global perspective of the data, it is apparent that metastatic PCA dominates the analysis and has the greatest variation in gene expression of the samples tested. Bars on the left or right of each matrix represent clusters of coordinately expressed genes highlighting interrelationships between specimens. For example, Clusters b3 and c1 represent genes down-regulated in both localized and metastatic PCA (Figures 1b and 1c). By contrast, Clusters b6 and b4 highlight genes that are specifically up- and down- regulated in metastatic PCA, respectively (Figure 1b). IGFBP-5, DAN1, FAT tumor suppressor and RAB5A are examples of genes that are down-regulated specifically in metastatic PCA and also have a proposed role in oncogenesis ("magnified" regions, Figure 1b). Similarly, cancer-related genes that are up-regulated in metastatic PCA include MTA-1 (metastasis-associated 1), MYBL2, and FLS353 (preferentially expressed in colorectal cancer). Many genes in this "met-specific" cluster are shared by both the metastatic PCA tissue and the two PCA cell lines DU145 and LnCAP.

Data was also obtained from the expression profiling of additional prostate tissue specimens profiled against a commercial prostate reference pool (CPP). A total of 53 prostate specimens were profiled against the commercial pool. They include 4 normal adjacent prostate tissue (NAP), 14 benign prostatic hyperplasia (BPH), 1 prostatitis, 14 localized prostate cancer (PCA) and 20 hormone refractory metastatic PCA (MET). Prior to hierarchial average-linkage clustering, the data was filtered for at least 3-fold change in Cy5/Cy3 ratios and measurements present in 75% of the samples. By this method 1325 genes were selected. The data expands on Figure 1c with an additional 40 samples, which include all from Figure 1b, and also includes 28 additional prostate specimens.

2. Focused Clusters

Data was next assessed by examining functional groups of known, named genes. Cancer-related functional clusters were arbitrarily defined including cell growth/cell death, cell adhesion, anti-protease/protease, free radical scavengers, inflammation/immunity, phosphatase/kinase, transcription, and miscellaneous (Figures 2 and 6).

One of several available methods of gene selection was used to create a more limited set of genes for future exploration. In one method, t-statistics (based on MET/PCA vs. benign) were computed for each gene. The cell line samples were excluded from the analysis. Also, genes and ESTs that had data missing from 20% of samples were excluded from analysis. The t-statistics were ranked in two ways. First, they were ranked by absolute magnitude, which takes into account the inter-sample variability in expression ratios. Second, they were ranked by the magnitude of the numerator of the test statistic, which is based on the biological difference in expression ratios and designated as "effect size" (for MET/PCA vs. benign). A scatterplot of the genes with the 200 largest effect sizes and 200 largest t-statistics was then plotted (See Figure 7). Figure 7 shows gene selection based on computed t-statistics for each gene. Two groups were used in the analysis: PCA/MET and benign (NAP/BPH). Figure 7a shows analysis of NAP pool data. Figure 7b shows analysis of CP pool data. Selected genes are named and 200 genes for each data set are shown. Gene selection based on

each method is shown. Selected gene names or symbols (as specified by Human genome organization (HUGO) gene nomenclature) are shown.

Genes that made both lists were also looked at separately in order to identify potential candidate genes. Implementing this methodology on both reference pool data sets (NAP and CP) yielded genes that included hepsin, pim-1, IM/ENIGMA, TIMP2, hevin, rig, and thrombospondin-1, among others. Several genes identified using gene selection methods are described in more detail in the context of "functional" clusters described in Figure 2.

Figure 2 shows the differential expression of functional clusters of select genes in prostate cancer. Gene names or symbols (as specified by Human genome organization (HUGO) gene nomenclature) are shown. The same convention for representing changes in transcript levels was used as in Figure 1. The sample order from Figure 1 was preserved for clarity.

Figure 8 shows a focused cluster of PCA-related genes. The same convention for representing changes in transcript levels was used as in Figure 1. This cluster of 231 genes was generated by selecting for a 3.5-fold variation in at least 2 of any class, and ratio measurements present in 75% of the samples. Classes included: PCA vs. NAP, MET vs. NAP, PCA vs. CP and MET vs. CP.

The reliability of the hierarchical clustering results was assessed using three separate methods: that of Calinski and Harabasz (1974), Hartigan (1975) and Krzanowski and Lai (1985). The number of "stable" clusters estimated by all these methods is two. In the CP pool data set, that would elicit a valid benign cluster (NAP and BPH) and a malignant cluster (PCA and MET).

Many of the genes identified in these "focused" clusters have been implicated directly or indirectly as cancer biomarkers or mediators of carcinogenesis. Several have been shown to be dysregulated in PCA. For example, the tumor suppressor gene PTEN was down-regulated, while the proto-oncogene myc was up-regulated in the microarray analysis of PCA (Figure 2) (Abate-Shen and Shen, *supra*). Likewise, decreased expression of E-cadherin and increased expression of fatty acid synthase, both of which have been shown to be dysregulated in PCA were observed (Tomita *et al.*, Cancer Res., 60:3650 [2000] and Shurbaji *et al.*, Hum. Pathol., 27:917 [1996]). In addition to

uncharacterized expressed sequence tags (ESTs), there are numerous genes that were identified by the screen but not previously known to be associated with PCA. It is contemplated that they find use as cancer markers.

Exemplary nucleic acid sequences for some of the genes identified in focused clusters are shown in Figures 9 and 10. The present invention is not limited to the particular nucleic acid sequences described in Figures 9 and 10 . One skilled in the art recognizes that additional variants, homologs, and mutants of the described sequences find use in the practice of the present invention.

3. Comparison Between NAP and CP pools

A direct comparison between the NAP and CP pool was also made and notable gene expression differences were readily apparent. Figure 5 shows a comparison between the NAP and CP pools. The same convention for representing changes in transcript levels was used as in Figure 1. The cluster was obtained by selecting for genes with at least a 2.5-fold variation in any two of the samples of each class, namely the normal tissues versus the NAP pool and normal tissue versus the CP pool at a 50% filter. Of the genes analyzed 59 were selected with this criteria. Genes that were found to be up-regulated in the NAP pool in comparison with CP pool included connective tissue growth factor, EGR-1 (Early Growth Response 1), matrilysin (MMP7), CFLAR/I-FLICE (caspase 8 and FADD-like apoptosis regulator), lumican, serum glucocorticoid regulated kinase, lens epithelium derived growth factor, PAI1 (plasminogen activator inhibitor type I), JUN and FOS B, among others. Vascular endothelial growth factor (VEGF), growth arrest specific (GAS1), cholecystokinin (CCK), amiloride binding protein (ABP1) were among the down-regulated genes in the normal adjacent prostate pool when compared to the commercial pool. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that the gene expression differences between normal prostate adjacent to PCA (NAP) and normal prostate tissue from individuals without prostate pathology (CP) may be attributable to a "field effect" induced by PCA itself.

Example 3

Northern Blot Analysis

Thirty micrograms of total RNA was resolved by denaturing formaldehyde agarose gel and transferred onto Hybond membrane (Amersham) by a capillary transfer set up. Hybridizations were performed by the method described by Church and Gilbert, 1984. Signal was visualized and quantitated by phosphorimager. For relative fold estimation, the ratio between the signals obtained from hepsin and GAPDH probes was calculated.

Selected genes identified by microarray analysis were corroborated by Northern analysis. For example, hevin, 4 1/2 LIM domain protein and gelsolin were shown to be 3.2-, 3.2- and 1.9- fold down-regulated, respectively by microarray and 8.8-, 4.5-, and 3.5- fold down-regulated by Northern analysis. Similarly, hepsin was 4.3-fold up-regulated by microarray and 11.3- fold up-regulated by Northern analysis (Figure 3a). As hepsin is a cell-surface serine protease with transcript expression precisely restricted to localized and metastatic PCA, its expression was examined in more detail at the protein level (See Example 4 below).

Example 4

Tissue Analysis

This example describes the analysis of protein expression in normal and cancerous prostate tissues.

A. Tissue microarray construction.

Kononen *et al.* have described a method for evaluating tumor tissues in large numbers on a single glass slide (Kononen *et al.*, Nat. Med., 4:844 [1998]). These high-density tissue microarrays allow for analysis of up to 1,000 tissue samples on a single slide. These slides can be evaluated by routine light microscopy on hematoxylin and eosin (H&E) prepared and immunohistochemically stained slides. Thus, candidate cancer biomarkers, identified by gene expression methodologies, can be evaluated at the protein level over a large number of clinically stratified tumor specimens.

Prostate tissues used in microarray analysis included 4 BPH, 8 NAP, 1 commercial pool of normal prostate tissue (from 19 individuals), 1 prostatitis, 11 localized PCA, and 7 metastatic PCA specimens. High-density tissue microarrays (TMA) were assembled using a manual tissue puncher/array (Beecher Instruments, Silver Springs, MD) as previously described (Kononen *et al.*, Nat. Med., 4:844 [1998]; Perrone *et al.*, J. Natl. Cancer Inst., 92:937 [2000]). The instrument consists of thin-walled stainless steel needles with an inner diameter of approximately 600μm and stylet used to transfer and empty the needle contents. The assembly is held in an X-Y position guide that is manually adjusted by digital micrometers. Small biopsies are retrieved from selected regions of donor tissue and are precisely arrayed in a new paraffin block. Tissue cores were 0.6 mm in diameter and ranged in length from 1.0 mm to 3.0 mm depending on the depth of tissue in the donor block. Multiple replicate core samples of normal, HGPIN, and PCA were acquired from each tissue block of each case. Cores were inserted into a 45 x 20 x 12 mm recipient bock and spaced at a distance of 0.8 mm apart. Prostate tumor grading was performed using the system described by Gleason (Gleason, Cancer Chemother Rep., 50:125 [1966]). Pathologic stages for the radical prostatectomies were determined using the TNM staging system (Schroder *et al.*, Prostate Suppl., 4:129 [1992]). Surgical margins were assessed separately and are not included in tumor staging.

B. Immunohistochemistry

TMA sections were cut at five- micron thick intervals for immunohistochemistry. Initial sections were stained for hematoxylin and eosin to verify histology. TMA slides prepared from formalin- fixed paraffin embedded tissue were heated for 0.5 - 1 hours at 60° centigrade. All slides were placed in 10 millimolar citrate buffer (pH 6.0) and microwaved for 5 minutes. Standard biotin-avidin complex immunohistochemistry was performed. The affinity purified polyclonal Rabbit antibody against hHepsin was used at a 1:40 dilution (original concentration 0.2 mg/ml) for this study. Immunostaining intensity was scored by a dedicated genitourinary pathologist as absent, weak, moderate, or strong. Scoring was performed using a telepathology system in a blinded fashion without knowledge of overall Gleason score (*e.g.*, tumor grade), tumor size, or clinical

outcome (Perrone *et al., supra*). A total of 738 tissue samples from benign (n=205), high- grade PIN (n=38), localized prostate cancer (n=335) and hormone refractory prostate cancer (n=160) were examined.

Similarly, pim-1 was analyzed using two TMA blocks from a total of 810 PCA samples from 135 patients. Six PCA samples were evaluated from each case and a median score was calculated. In addition, a small number of samples with benign prostatic tissues (*e.g.*, benign epithelium and atrophy) and HG-PIN were examined. Immunohistochemistry was performed as above, using a rabbit polyclonal antibody against the N-terminus of pim-1 (Santa Cruz Biotechnology) at a 1:100 dilution. Pim-1 demonstrated cytoplasmic staining and was graded as either negative, weak, moderate, or strong. All samples were reviewed blinded with respect to all related pathology and clinical data.

C. Statistical methods

A nonparametric ANOVA test (Mann-Whitney [two categories]) was employed to evaluate whether the prostate samples expressed hepsin and pim-1 at different levels based on various parameters (tissue type, Gleason score, and tumor size). Kaplan-Meier analysis was used to estimate the cumulative percentage of PSA free progression ("survival"). The log-rank test was employed to assess the differences in disease free progression hepsin immunostaining. Cox proportional- hazard regression was used for multivariate analysis. Commercial software from SPSS (Chicago, IL) was used for this study.

D. Results

1. Hepsin

Microarrays used in this study are shown in Figure 3b. Over 700 benign and malignant prostate tissues were immunohistochemically profiled on tissue microarrays (Figure 3c-e) using an affinity-purified hepsin-peptide antibody (Tsuji *et al.*, J. Biol. Chem., 266:16948 [1991]). Figure 3 shows the overexpression of Hepsin, a transmembrane serine protease, in prostate cancer. Figure 3a shows a Northern blot

analysis of human hepsin (top) and normalization with GAPDH (bottom). NAT indicates normal adjacent prostate tissue and PCA indicates prostate cancer. Figure 3b shows tissue microarrays used for hepsin analysis. Staining was done with hemotoxylin and eosin to verify histology.

Immunohistochemical stains demonstrated absent or weak staining of benign prostate (c1), strong staining in localized prostate cancer (c2-6), and strong staining in a high-grade prostate tumor (magnification 100X was used for all images, samples measure 0.6 mm in diameter). Benign prostate glands demonstrate weak expression in the secretory, luminal cells and strong basal cell staining. In areas where prostate cancer and benign prostate glands are seen, significant hepsin staining differences are observed. Infiltrating prostate cancers (d3-4) demonstrate strong hepsin protein expression. Magnification for all images was 400X. Figure 3c shows a histogram of hepsin protein expression by tissue type. Benign prostate hyperplasia (BPH). High-grade intraepithelial neoplasia (HG-PIN). Localized prostate cancer (PCA). Hormone-refractory prostate cancer (MET). Relative strength of hepsin staining was qualitatively assessed and categorized. Percentage of hepsin staining per category is shown on the y-axis. Figure 3d shows Kaplan Meier Analysis. PSA-free survival was stratified by level of hepsin protein expression into two categories absent/low expression (circles) versus moderate/strong expression (squares).

Internal controls showed that liver tissue, as previously described, stained strongly for hepsin. Overall, hepsin exhibited predominantly membrane staining and was preferentially expressed in neoplastic prostate over benign prostate (Mann-Whitney test, $p<0.0001$). Importantly, the precursor lesion of PCA, HG-PIN, had the strongest expression of hepsin, and almost never had absent staining (Mann-Whitney, $p<0.0001$). Most cases of low or absent hepsin staining were seen in benign prostate specimens. In addition, hormone refractory metastatic cancers were intermediate in staining intensity between localized prostate tumors and benign prostate.

Men who develop elevated PSA levels following radical prostatectomy are at a high risk to develop distant metastases and die due to prostate cancer (Pound *et al.*, JAMA, 281:1591 [1999]. Therefore, to assess the usefulness of hepsin as a potential PCA biomarker, PSA failure was defined as a PSA elevation of greater than 0.2 ng/ml

following radical prostatectomy. Analysis was performed on 334 localized prostate cancer samples treating each as an independent sample. PSA elevation following radical prostatectomy was significantly associated with absent and low hepsin immunostaining with a 28% (46/119 samples) PSA failure rate, in contrast to 17% (28/141 samples) PSA failure rate for tumors with moderate to strong hepsin expression (Figure 3d, Log Rank test P=0.03). Multivariate analysis was performed to examine if these results were independent of Gleason score, a well-established histologic grading system for PCA (Gleason, Hum. Pathol., 23:273 [1992]). Based on the results from fitting a Cox proportional hazards model, there is an association of weak or absent hepsin protein expression in PCA with increased risk of PSA elevation following prostatectomy, similar to high Gleason score (corresponding risk ratios were 2.9 (p=0.0004) and 1.65 (p=0.037), respectively). Weak or absent hepsin expression was also associated with large prostate cancers; the median tumor dimension for prostate tumors with moderate to strong expression was 1.3 cm but 1.5 cm for tumors with absent or weak staining (Mann-Whitney Rank test, P=0.043). Taken together, hepsin protein expression in PCA correlated inversely with measures of patient prognosis.

Hepsin is a 51 kDa transmembrane protein with highest expression in the liver, and like PSA, is a serine protease (Kurachi *et al.*, Methods Enzymol., 244:100 [1994]). The protease domain of hepsin has access to the extracellular space and can potentially activate other proteases or degrade components of extracellular matrix. The function of hepsin is poorly understood. It has been proposed to have a role in controlling cell growth (Torres-Rosado *et al.*, PNAS, 90:7181 [1993], cell morphology, and activating the extrinsic coagulation pathway on the cell surface, leading to thrombin formation (Kazama *et al.*, J. Biol. Chem., 270:66 [1995]). Additionally, hepsin mRNA levels have been shown to be elevated in ovarian carcinomas (Tanimoto *et al.*, Cancer Res., 57:2884 [1997]). The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that the high expression of hepsin in HG-PIN, and not benign prostate, suggests that hepsin plays a role in the establishment of PIN or in the transition from HG-PIN to carcinoma. Subsequent decreases in hepsin expression seen in large localized cancers and hormone-refractory cancers suggest a decreased requirement

of this protease in later stages of PCA. Alternatively, patients with advanced PCA often develop disseminated intravascular coagulation (DIC) (Riddell *et al.*, J. Nucl. Med., 37:401 [1996]) whereby hepsin may play an important role.

2. pim-1

Tumorigenic growth of the prostate depends on the evasion of normal homeostatic control mechanisms, where cell proliferation exceeds cell death (Bruckheimer and Kyprianou, Cell Tissue Res., 301: 153 [2000]). While it is well known that the oncogene myc is overexpressed in many PCAs (Buttyan *et al.*, prostate 11:327-37 [1987]; Abate-Shen and Shen, *supra*), the present invention demonstrates that the proto-oncogene pim-1 kinase is similarly up-regulated (cell growth/cell death cluster, Figure 2). Previous studies suggest that the cooperative interaction between pim-1 and myc may induce lymphoid cell transformation by promoting cell cycle progression and blocking apoptosis (Shirogane, *et al.*, Immunity 11:709 [1999]). The present analysis supports a similar co-transcriptional regulation (or gene amplification) of pim-1 and myc possibly mediating a synergistic oncogenic effect in PCA.

Pim-1 kinase protein expression in PCA was also explored using high-denisty TMAs. Figure 4 shows the overexpression of pim-1 in prostate cancer. Immunohistochemical stains demonstrated absent or weak staining of benign prostate, and strong cytoplasmic staining in localized prostate cancer. Benign prostate glands demonstrated absent or weak expression in the secretory, luminal cells. Infiltrating prostate cancers demonstrated strong pim-1 protein expression. Magnification for all images 1000X. Figure 4a shows a histogram of pim-1 protein expression by tissue type as assessed from 810 tissue microarray elements. High-grade intraepithelial neoplasia (HG-PIN). Localized prostate cancer (PCA). Relative strength of pim-1 staining is represented in the included legend. The percentage of pim-1 staining per category shown on y-axis. Figure 4b shows Kaplan-Meier analysis demonstrating that patients with PCA that have negative to weak pim-1 expression (bottom line) are at a greater risk of developing PSA-failure following prostatectomy (log rank p=0.04). PSA-free survival was stratified by level of pim-1 protein expression into two categories absent/weak expression (bottom line) versus moderate/strong expression (top line).

Pim-1 protein was found to be markedly overexpressed in PCA (Figure 4). Negative to weak pim-1 protein expression was observed in the majority of benign prostatic epithelial (97%), prostatic atrophy (73%), and high-grade PIN (82%) samples (Figure 4a). In contrast, moderate to strong pim-1 expression was observed in approximately half of the PCA samples (51%) (Figure 4a). Kaplan-Meier analysis for PSA- free survival demonstrated positive extraprostatic extension, seminal vesicle invasion, Gleason score greater than 7 and decreased pim-1 expression to be associated with a higher cumulative rate of PSA failure (Figure 4b). By univariate Cox models, it was found that Pim-1 expression is a strong predictor of PSA recurrence (hazard ratio (HR)= 2.1 (95% CI 1.2-3.8, p=0.01)).

Among the variables examined, significant predictors of PSA recurrence were Gleason score (HR=1.8 (95% CI 1.1-3.0), p=0.03), Gleason pattern 4/5 PCA (HR=3.9(95%CI 1.8-8.3), p<0.001), extraprostatic extension status (HR=2.6 (95%CI 1.6-4.2), p<0.0001), surgical margin status (HR=2.6 (95%CI 1.2-5.6), p=0.01), seminal vesicle status (HR=3.5 (95%CI 2.0-6.2), p<0.0001), the natural log of pre-operative PSA level (HR=2.5 (95%CI 1.6-3.8), p<0.001), HR=2.4, p<0.001), and maximum tumor dimension (HR=2.7 (95%CI 1.6-4.7), p<0.0001). Presence of Gleason pattern 4/5 PCA (HR=3.8 (95%CI 1.4-10.0), p<0.01), Ln(PSA) (HR=2.1 (95%CI 1.1-3.9), p=0.02), and decreased pim-1 protein expression (HR=4.5 (95%CI 1.6-15.2), p=0.01) were both found to be significant predictors of PSA recurrence by a multivariate Cox model. Thus, even more so than hepsin, decreased expression of pim-1 kinase in PCA correlated significantly with measures of poor patient outcome.

Pim-1 kinase is a proto-oncogene that is regulated by cytokine receptors (Matikainen *et al.*, Blood 93:1980 [1999]). It was first described as a common site of proviral integration in murine retrovirus-induced T cell lymphomas (Cuypers *et al.*, Cell 37:141 [1984]), and was previously thought to be involved exclusively in hematopoietic malignancies (Breuer *et al.*, Nature 340:61 [1989]). Co-transcriptional regulation of pim-1 and myc was observed in the experiments described herein (Figure 2 cell growth/cell death cluster). Chronic overexpression of myc in the ventral prostate of transgenic mice induced epithelial abnormalities similar to low-grade PIN, but progression to adenocarcinoma in this model was never observed (Zhang *et al.*, Prostate 43:278 [2000]).

The present invention is not limited to any one mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that pim-1 overexpression may potentiate myc- induced prostate carcinogenesis.

Figure 8 provides a schematic overview of representative genes differentially expressed in PCA identified by DNA microarray analysis. Genes are grouped functionally and arrows represent up- or down- regulation in metastatic hormone-refractory PCA (MET) and/or localized PCA (PCA) relative to normal prostate epithelium. See Figure 2 for gene expression levels.

Example 5

AMACR Expression Analysis

The Example describes the analysis of the gene expression data described in Examples 1-4 above to identify AMACR as being consistently over-expressed in prostate cancer.

A. Tissue Samples

In order to examine the widest range of prostate cancer specimens, clinical samples were taken from the radical prostatectomy series at the University of Michigan and from the Rapid Autopsy Program. Both programs are part of the University of Michigan Prostate Cancer Specialized Program of Research Excellence (S.P.O.R.E.) Tissue Core.

Prostatectomy cases for the tissue microarray (TMA) outcomes array were selected from a cohort of 632 patients, who underwent radical retropubic prostatectomy at the University of Michigan as a monotherapy (*i.e.*, no hormonal or radiation therapy) for clinically localized prostate cancer between the years of 1994 and 1998. Clinical and pathology data for all patients was acquired with approval from the Institutional Review Board at the University of Michigan. Detailed clinical, pathology, and TMA data is maintained on a secure relational database (Manley *et al.*, Am. J. Pathol., 159:837 [2001]).

Processing of the prostate specimens began within approximately 15-20 minutes after surgical resection. The prostates were partially sampled and approximately 50% of the tissue was used for research. This protocol has been evaluated in a formal study to assure that partial sampling does not impair accurate staging and evaluation of the surgical margins (Hollenbeck *et al.*, J. Urol., 164:1583 [2000]). Briefly, alternate sections of the prostate gland were submitted for histologic review. The remaining sections were frozen and stored in the SPORE Tissue Core. These samples were collected only from patients who had signed an IRB-approved informed consent. The samples were snap-frozen in OCT embedding media at -80°C and stored in a holding area until the pathology report was finalized. These frozen samples were not available to researchers until adequate diagnosis and staging had been performed. The samples used for cDNA expression array analysis and RT-PCR were all evaluated by the study pathologists. All samples were grossly trimmed such that greater than 95% of the sample represented the desired lesion (*e.g.*, prostate cancer, BPH, or benign prostate). Samples with prostate cancer were also assigned a Gleason score based on the sample used for molecular analysis.

In order to study hormone refractory prostate cancer, a Rapid Autopsy Protocol was used, which represents a valuable source of metastatic prostate tumors. Modeled after protocols developed at the University of Washington (Seattle, WA.) and Johns Hopkins University (Baltimore, MD), this program allows men with advanced prostate cancer to consent to an autopsy immediately after death. To date, 23 complete autopsies have been performed with a median time of 2 hours from death to autopsy. This procedure has previously been described in detail (Rubin *et al.*, Clin. Cancer Res., 6:1038 [2000]). In brief, patients diagnosed with hormone refractory prostate cancer were asked to take part in a posthumous tissue donor program. The objectives and procedures for tissue donation were explained to the patient. Having agreed to participate in this IRB-approved tumor donor program, permission for autopsy is obtained before the death, with consent provided by the patient, or by next of kin. Hormone refractory primary and metastatic prostate cancer samples were collected using liquid nitrogen. Mirrored samples from the same lesion were placed in 10% buffered formalin. The fixed samples were embedded in paraffin and used for the development of TMAs. As with the

prostatectomy samples, the study pathologist reviewed the glass slides, circled areas of viable prostate cancer, while avoiding areas of necrosis, and used these slides as a template for TMA construction.

B. Pathology and Evaluation

Prostates were inked before the assessment of surgical margins. Surgical margins from the apex and base were cut perpendicular to the prostatic urethral axis. The seminal vesicles were cut perpendicular to their entry into the prostate gland and submitted as the seminal vesicle margin. The prostates for this study were all partially embedded, taking alternate full sections from the apex, mid, and base. Detailed prostatectomy pathology reports included the presence or absence of surgical margin involvement by tumor (surgical margin status), the presence of extraprostatic extension, and seminal vesicle invasion. Tumors were staged using the TNM system, which includes extraprostatic extension and seminal vesicle invasion but does not take into account surgical margin status (Bostwick *et al.*, Simin. Urol. Oncol., 17:222 [1999]). Tumors were graded using the Gleason grading system (Gleason, Cancer Chemother. Rep., 50:125 [1966]; Gleason, The Veterans Administration Cooperative Urological Research Group. Histologic Grading and Clinical Staging of Prostate Carcinoma. In: Tannenbaum M, editor. Urologic Pathology: The Prostate. Philadelphia: Lea & Febiger; 1977. p. 171-98).

As preparation for the construction of the TMAs, all glass slides were re-reviewed to identify areas of benign prostate, prostatic atrophy, high-grade prostatic intraepithelial neoplasia, and prostate cancer. To optimize the transfer of these designated tissues to the arrays, area of tumor involvement was encircled on the glass slide template as tightly around each lesion as possible. Areas with infiltrating tumor adjacent to benign glands were avoided.

C. RT-PCR

Total RNA integrity was judged by denaturing-formaldehyde agarose gel electrophoresis. cDNA was prepared using 1 μg of total RNA isolated from prostate tissue specimens. Primers used to amplify specific gene products were: AMACR sense, 5' CGTATGCCCCGCTGAATCTCGTG-3' (SEQ ID NO:100); AMACR antisense, 5'-

TGGCCAATCATCCGTGCTCATCTG-3' (SEQ ID NO:101); GAPDH sense, 5'-CGGAGTCAACGGATTTGGTCGTAT-3' (SEQ ID NO:102); and GAPDH antisense, 5'-AGCCTTCTCCATGGTGGTGAAGAC -3' (SEQ ID NO:103). PCR conditions for AMACR and GAPDH comprised 94°C for 5 min, 28 cycles of 95°C for 1 min, 60°C for 1 min (annealing), and 72°C for 1min, and a final elongation step of 72°C for 7 min. PCR reactions used a volume of 50 µl, with 1 unit of Taq DNA polymerase (Gibco BRL). Amplification products (5 µl) were separated by 2% agarose gel electrophoresis and visualized by ultraviolet light.

D. Immunoblot Analysis

Representative prostate tissue specimens were used for Western blot analysis. Tissues were homogenized in NP-40 lysis buffer containing 50 mmol/L Tris-HCl, pH 7.4, 1% Nonidet P-40 (Sigma, St. Louis. MO) and complete proteinase inhibitor cocktail (Roche, IN, USA). Fifteen µg of protein extracts were mixed with SDS sample buffer and electrophoresed onto a 10% SDS-polyacrylamide gel under reducing conditions. The separated proteins were transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ). The membrane was incubated for 1 hour in blocking buffer (Tris-buffered saline with 0.1% Tween (TBS-T) and 5% nonfat dry milk). The AMACR antibody (Obtained from Dr. R Wanders, University of Amsterdam) was applied at 1:10,000 diluted in blocking buffer overnight at 4°C. After washing three times with TBS-T buffer, the membrane was incubated with horseradish peroxidase-linked donkey anti-rabbit IgG antibody (Amersham Pharmacia Biotech, Piscataway, NJ) at 1:5000 for 1 hour at room temperature. The signals were visualized with the ECL detection system (Amersham Pharmacia biotech, Piscataway, NJ) and autoradiography.

For ß-tubulin western blots, the AMACR antibody probed membrane was stripped with Western Re-Probe buffer (Geno-tech, St. Louis, MO) and blocked in Tris-buffered saline with 0.1% Tween (TBS-T) with 5% nonfat dry milk and incubated with rabbit anti ß-tubulin antibodies (Santa Cruz Biotechnologies, Santa Cruz, CA) at 1:500 for two hours. The western blot was then processed as described above.

E. Immunohistochemistry

Standard indirect immunohistochemistry (IHC) was performed to evaluate AMACR protein expression using a polyclonal anti-AMACR antibody. Protein expression was scored as negative (score=1), weak (score 2), moderate (3) and strong (4). In order to evaluate whether AMACR protein expression was associated with prostate cancer proliferation, a subset of samples were evaluated using the monoclonal mouse IgG Mib-1 antibody for Ki-67 (1:150 dilution, Coulter-Immunotech, Miami, Fl). Microwave pretreatment (30 minutes at 100 C in Tris EDTA Buffer) for antigen retrieval was performed using 3,3' diaminobenzidine tetrahydrocloride as a chromogen. Lymph node tissue with known high Ki-67 positivity was used as a control.

F. Tissue Microarray Construction, Digital Image Capture, and Analysis

Five TMAs were used for this study. Three contained tissue from the prostatectomy series and two contained hormone refractory prostate cancer from the Rapid Autopsy Program. The TMAs were assembled using the manual tissue arrayer (Beecher Instruments, Silver Spring, MD) as previously described (Kononen *et al.*, Nat. Med., 4:844 [1998]; Perrone *et al.*, J. Natl. Cancer Inst., 92:937 [2000]). Tissue cores from the circled areas (as described above) were targeted for transfer to the recipient array blocks. Five replicate tissue cores were sampled from each of the selected tissue types. The 0.6 mm diameter TMA cores were each spaced at 0.8 mm from core-center to core-center. After construction, 4 μm sections were cut and H&E staining was performed on the initial slide to verify the histology.

TMA H&E images were acquired using the BLISS Imaging System (Bacus Labs, Lombard, IL). AMACR protein expression was evaluated in a blinded manner. All images were scored for AMACR protein expression intensity. In addition, all TMA samples were assigned a diagnosis (*i.e.*, benign, atrophy, high-grade prostatic intraepithelial neoplasia, and prostate cancer). This is recommended because the targeted tissues may not be what were actually transferred. Therefore, verification was performed at each step. TMA slides were evaluated for proliferation index using a CAS200 Cell Analysis System (Bacus Labs). Selected areas were evaluated under the 40X objective. Measurements were recorded as the percentage of total nuclear area that was positively

stained. All positive nuclear staining, regardless of the intensity, was measured. Sites for analysis were selected to minimize the presence of stromal and basal cells; only tumor epithelium was evaluated. Specimens were evaluated for Ki-67 expression as previously described (Perrone *et al.*, J. Natl. Cancer Inst. 92:937 [2000]). Each measurement was based on approximately 50–100 epithelial nuclei. Due to the fixed size of TMA samples, 5 repeat non-overlapping measurement was the maximum attainable.

G. Analysis of Prostate Needle Biopsies

In order to evaluate the usefulness of AMACR expression in diagnostic 18 gauge needle biopsies, 100 consecutive biopsies with prostate cancer or atypia that required further work-up were tested for AMACR expression. All cases were immunostained using two basal cell specific markers (34βE12 and p63) and AMACR. Cases were evaluated for cancer sensitivity and specificity. Twenty-six of these cases were seen in consultation with a pathologist and were considered diagnostically difficult, requiring expert review and additional characterization.

H. Results

Figure 11 shows a schematic of the DNA and tissue microarray paradigm that lead to the discovery and characterization of AMACR in prostate cancer. A) Prostate cancer progression as adapted from Abate-Shen and Shen, (Genes Dev., 14:2410 [2000]). Distinct molecular changes occur at each stage of prostate cancer progression that can be studied using DNA microarray or "chip" technology. B) cDNA generated from tumor (prostate cancer) and reference (benign prostate tissue) samples is labeled with distinguishable fluorescent dyes and interrogated with a DNA microarray that can monitor thousands of genes in one experiment. C) After hybridization, the DNA microarray is analyzed using a scanner and fluorescence ratios determined for each gene (in this case prostate cancer/ benign tissue). D) The ratios are deposited into a computer database and subsequently analyzed using various statistical algorithms. One exemplary method of surveying the data (Eisen *et al.*, PNAS 95:14863 [1998]) assigns color intensity to the ratios of gene expression. In this case, shades of red represent genes that are up-regulated in prostate cancer (*e.g.*, a ratio of 4.0) and shades of green represent

genes that a down-regulated (*e.g.*, ratio of 0.1). Genes that are unchanged between tumor and benign tissues are represented by a black color and missing elements by a gray color. E) Genes that are identified by DNA microarray can then be validated at the transcript level (*e.g.*, Northern blot, RT-PCR) or at the protein level (*e.g.*, immunoblot). F) Construction of prostate cancer tissue microarrays facilitates the study of hundreds of patients (rather than hundreds of genes). G) Each tissue microarray slide contains hundreds of clinically stratified prostate cancer specimens linked to clinical and pathology databases (not shown). H) Tissue microarray slides can then be analyzed using various molecular or biochemical methods (in this case immunohistochemistry). I) Both DNA and tissue microarray data have clinical applications. Examples include, but are not limited to: 1. using gene expression profiles to predict patient prognosis, 2. identification of clinical markers and 3. development of novel therapeutic targets.

Figure 12 summarizes AMACR transcript levels as determined by DNA microarray analysis over 57 prostate cancer specimens. Samples (Dhanasekaran *et al.*, Nature 412: 822 [2001]) were grouped according to tissue type and averaged. The experimental sample was labeled in the Cy5 channel while the reference sample (pool of benign prostate tissue) was labeled in the Cy3 channel. The box-plot demonstrates the range of AMACR expression within each group. Tissues were grouped into the following classes benign (normal adjacent prostate tissue), benign prostatic hyperplasia (BPH), clinically localized prostate cancer, and metastatic prostate cancer. In relation to benign prostate tissues, localized prostate cancer and metastatic prostate cancer were 3.1 (Mann-Whitney test, $p<0.0001$)and 1.67 (Mann-Whitney test, $p<0.004$) fold up-regulated, respectively (represented as Cy5/Cy3 ratios).

DNA microarray results of AMACR mRNA levels were validated using an independent experimental methodology. AMACR-specific primers were generated and RT-PCR performed on the various RNA samples from 28 prostate tissue specimens and 6 prostate cell lines (Figure 13A). GAPDH served as the loading control. Pool, refers to RNA from normal prostate tissues obtained from a commercial source. NAP, normal adjacent prostate tissue from a patient who has prostate cancer. 3+3, 3+4, 4+4, refers to the major and minor Gleason patterns of the clinically localized prostate cancer (PCA) examined. MET, metastatic prostate cancer. Various prostate cell lines are also

examined. RT-PCR without enzyme served as a negative control. An RT-PCR product was clearly observed in the 20 localized prostate cancer samples but not in the benign samples examined. Metastatic prostate cancer and prostate cell lines displayed varying levels of AMACR transcript as compared to localized prostate cancer.

In order to gauge AMACR protein levels, immunoblot analysis was performed on selected prostate tissue extracts (Figure 13B). β-tubulin served as a control for sample loading. Similar to AMACR transcript, over-expression of AMACR protein was observed in malignant prostate tissue relative to benign prostate tissue.

In order to validate protein expression of AMACR *in situ*, a separate cohort of prostate samples from those used in the cDNA expression array analysis was used. These prostate samples were taken from the University of Michigan Prostate SPORE Tissue Core and were assembled onto high-density tissue microarrays (schematically illustrated in Figure 11F-H). Moderate to strong AMACR protein expression was seen in clinically localized prostate cancer samples with predominately cytoplasmic localization. A large contrast in levels of AMACR in malignant epithelia relative to adjacent benign epithelia was seen. Prostatic intraepithelial neoplasia (PIN) and some atrophic lesions, which are thought to be potentially pre-cancerous lesions (Putzi *et al.*, Urology 56:828 [2000]; Shah *et al.*, Am. J. Pathol., 158:1767 [2001]), demonstrated cytoplasmic staining of AMACR. High-grade prostate cancer also demonstrated strong cytoplasmic staining. However, no association was identified with AMACR staining intensity and Gleason (tumor) score. Many of the metastatic prostate cancer samples demonstrated only weak AMACR expression. The metastatic samples showed uniform PSA immunostaining, confirming the immunogenicity of these autopsy samples.

In order to assess AMACR protein expression over hundreds of prostate specimens, the tissue microarray data was quantitated. Benign prostate, atrophic prostate, PIN, localized prostate cancer, and metastatic prostate cancer demonstrated mean AMACR protein staining intensity of 1.0 (SE 0), 2.0 (SE 0.1), 2.5 (SE 0.1), 3.0 (SE 0), and 2.5 (SE 0.1), respectively (ANOVA p-value<0.0001). This data is graphically summarized using error bars representing the 95% confidence interval for each tissue category (Figure 14).

The correlation of AMACR levels with tumor proliferation was next investigated using Ki-67 (Perrone *et al., supra*). There was no significant association between AMACR expression and Ki-67 expression with a correlation coefficient of 0.13 (p-value=0.22). In addition, no significant associations were identified between AMACR protein expression and pathology parameters such as radical prostatectomy, Gleason score, tumor stage, tumor size (cm), or surgical margin status. AMACR protein levels were next evaluated for association with PSA recurrence following surgery in 120 prostatectomy cases with a median follow-up time of 3 years. No statistically significant association was identified. AMACR demonstrated uniform moderate to strong expression in clinically localized prostate cancer with a high sensitivity for tumor and an equally high specificity. In addition, a preliminary survey of normal tissues including ovary, liver, lymph nodes, spleen, testis, stomach, thyroid, colon, pancreas, cerebrum, and striated muscle revealed significant AMACR protein expression in only normal liver.

The large difference in AMACR protein levels between normal secretory epithelial cells and malignant cells provides a clinical use for testing AMACR expression in prostate needle biopsy specimens. In diagnostically challenging cases, pathologists often employ the basal cell markers 34βE12 (O'Malley *et al.*, Virchows Arch A Patho. Anat. Histopathol., 417:191 [1990]; Wojno *et al.*, Am. J. Surg. Pathol., 19:251 [1995]; Googe *et al.*, Am. J. Clin. Pathol., 107:219 [1997] or p63 (Parson *et al.*, Urology 58:619 [2001]; Signoretti *et al.*, Am. J. Pathol., 157:1769 [2000]), which stain the basal cell layer of benign glands. This second basal cell layer is absent in malignant glands. In many equivocal biopsy specimens, the surgical pathologist must rely on absence of staining to make the final diagnosis of prostate cancer. The clinical utility of AMACR immunostaining on 94 prostate needle biopsies was evaluated. The results are shown in Table 2. The sensitivity and specificity were calculated as 97% and 100%, respectively. These results included 26 cases where the final diagnosis required the use of a basal cell specific immunohistochemical marker (*i.e.*, 34βE12 or p63).

This example demonstrated that AMACR is associated with PCA and that AMACR expression in prostate biopsies is useful for the diagnosis of cancer in inconclusive biopsy samples.

Table 2

Clinical utility of Assessing AMACR Protein in Prostate Needle Biopsies (n=94)

Sensitivity (TP/(TP+FN))	Specificity (TN/(TN+FP))	Positive Predictive Value (TP/(TP+FP))	Negative Predictive Value (TN/(TN+FN))
97% ((68/(2+68))	100% ((24/(24+0))	100% ((68/(68+0))	92% ((24/24+2))

Example 6

Hormone Regulation of AMACR

This example describes studies that indicate that AMACR expression is hormone independent.

A. Sample collection, cDNA array and TMA construction and evaluation

Clinical samples were taken from the radical prostatectomy series and from the Rapid Autopsy Program at the University of Michigan. Both are part of the University of Michigan Prostate Cancer Specialized Program of Research Excellence (S.P.O.R.E.). Primary PCA of metastatic cases as well as lymph node metastases were contributed in collaboration from the University of Ulm, Germany. Detailed clinical and expression analysis as well as TMA data was acquired and maintained on a secure relational database according to the Institutional Review Board protocol of both institutions. Tissue procurement for expression analysis on the RNA level is described in the above examples. For the development of TMA, samples were embedded in paraffin. The study pathologist reviewed slides of all cases and circled areas of interest. These slides were used as a template for construction of the six TMAs used in this study. All TMAs were assembled using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). At least three tissue cores were sampled from each donor block. Histologic diagnosis of the tissue cores was verified by standard haematoxylin and eosin (H&E) staining of the initial TMA slide. Standard biotin-avidin complex immunohistochemistry (IHC) was performed using a polyclonal anti-AMACR antibody (Ronald Wanders, University of Amsterdam). Digital images were acquired using the BLISS Imaging System (Bacus Lab, Lombard, IL). Staining intensity was scored as negative (score=1), weak (score 2), moderate (3) and strong (4). For exploration of the treatment effect by the means of

hormonal withdrawal before radical prostatectomy, standard slides were used for regular H&E staining and consecutive sections for detection of AMACR expression. In order to test AMACR expression in poorly differentiated colon cancers, cases were used from a cohort of well-described colon tumors. In addition to well-differentiated colon cancers, a recently described subset of poorly differentiated colon carcinomas with a distinctive histopathological appearance, termed large cell minimally differentiated carcinomas, was used. These poorly differentiated colon carcinomas had a high frequency of the microsatellite instability phenotype.

B. Cell culture and immunoblot analysis

Prostate cell lines (RWPE-1, LNCaP , PC3 and DU145) were obtained from the American Tissue Culture Collection. Cells were maintained in RPMI-1640 with 8% decomplemented fetal bovine serum, 0.1% glutamine and 0.1% penicillin and streptomycin (BioWhittaker, Walkersville, MD). Cells were grown to 75% confluence and then treated for 24 and 48 with the antiandrogen bicalutamide (CASODEX, Zeneca Pharmaceuticals, Plankstadt, Germany) at a final concentration of 20 μM or with methyltrienolone (synthetic androgen (R1881); NEN, Life Science Products, Boston, MA) at a final concentration of 1 nM. Cells were harvested and lysed in NP-40 lysis buffer containing 50 mmol/L Tris-HCl, pH 7.4, 1% Nonidet P-40 (Sigma, St. Louis, MO) and complete proteinase inhibitor cocktail (Roche, IN, USA). 15 μg of protein extracts were mixed with SDS sample buffer and electrophoresed onto a 10% SDS-polyacrylamide gel under reducing conditions. After transferring, the membranes (Amersham Pharmacia Biotech, Piscataway, NJ) were incubated for 1 hour in blocking buffer (Tris-buffered saline with 0.1% Tween and 5% nonfat dry milk). The AMACR antibody was applied at 1:10.000 diluted blocking buffer overnight at 4°C. After three washes with TBS-T buffer, the membrane was incubated with horseradish peroxidase-linked donkey anti-rabbit IgG antibody (Amersham Pharmacia Biotech, Piscataway, NJ) at 1:5000 for 1 hour at room temperature. The signals were visualized with the ECL detection system (Amersham Pharmacia biotech, Piscataway, NJ). For ß-tubulin blots, membranes were stripped with Western Re-Probe buffer (Geno-tech, St. Louis, MO) and blocked in Tris-buffered saline with 0.1% Tween with 5% nonfat dry milk and incubated

with rabbit anti ß-tubulin antibodies (Santa Cruz Biotechnologies, Santa Cruz, CA) at 1:500 for two hours. For PSA expression the membranes were reprobed in the described manner with PSA antibody (rabbit polyclonal; DAKO Corporation, Carpinteria, CA) at 1: 1000 dilution and further processed.

C. Statistical Analysis

Primary analysis of the cDNA expression data was done with the Genepix software. Cluster analysis with the program Cluster and generation of figures with TreeView was performed as described above. AMACR protein expression was statistically evaluated using the mean score result for each prostate tissue type (*i.e.*, benign prostate, naive localized or advanced prostate cancer, hormone treated and hormone refractory prostate cancer). To test for significant differences in AMACR protein expression between all tissue types, a one-way ANOVA test was performed. To determine differences between all pairs, a post-hoc analysis using the Scheffé method was applied as described above. For comparison of naive primaries to their corresponding lymph node metastases with respect to AMACR protein expression, a non parametric analysis (Mann Whitney test) was performed. To compare AMACR expression intensity to the scored hormonal effect of the pretreated localized prostate cancer cases the Mantel-Haenszel Chi-Square test was applied. AMACR expression scores are presented in a graphical format using error-bars with 95% confidence intervals. P-values <0.05 were considered statistically significant.

D. Results

Hierarchical clustering of 76 prostate tissues including benign, BPH, localized PCA and metastatic PCA and filtering for only those genes with a 1.5 fold expression difference or greater, clustered the samples into histologically distinct groups as described above. As demonstrated by a TreeView presentation of this data (Figure 15), AMACR was one of several genes that demonstrated over expression at the cDNA level of PCA samples with respect to benign pooled prostate tissue. The highest level of over expression by cDNA analysis was in the clinically localized PCA cases.

In order to further investigate the role of AMACR protein expression in samples with variable differentiation and exposure to anti-androgen treatment, several TMAs with a wide range of PCA were constructed: a total of 119 benign prostate samples, 365 primary hormone naive PCA samples, 37 naive prostate cancer lymph node metastases, and 41 hormone refractory metastatic PCA samples were evaluated. An additional 49 hormone treated primary prostate cancers (including 22 on standard slides) were examined for histologic changes associated with anti-androgen treatment and AMACR protein expression. The mean AMACR protein expression levels for each tissue category is presented in Figure 16. Benign prostate, naive primary prostate cancer, hormone treated primary cancer, and hormone refractory metastatic tissue had a mean staining intensity of 1.28 (Standard Error SE 0.038, 95% Confidence Intervals CI 1.20-1.35), 3.11(SE 0.046, CI 3.02-3.20), 2.86 (SE 0.15, CI 2.56-3.15) and 2.52 (SE 0.15, CI 2.22-2.28), respectively). One-way ANOVA analysis revealed a p-value of <0.0001. To specifically examine the difference between different tissue types, a post-hoc pair-wise comparison was performed. Clinically localized PCA demonstrated a significantly stronger AMACR protein expression as compared to benign prostate tissue (post-hoc analysis using Scheffé method, mean difference =1.83, $p<0.0001$, CI 1.53-2.13). A significant decrease in AMACR protein expression was observed in the metastatic hormone refractory PCA samples with respect to clinically localized PCA (0.59, $p=0.002$, CI 0.15-1.03). Hormone treated primaries had a mean AMACR expression of 2.86, which was between the expression levels of naive primaries (3.11) and hormone refractory cases (2.52) (post-hoc analysis using Scheffé method, $p=0.51$, CI –0.66-0.16 and $p=0.56$, CI –0.23-0.91). There was no significant difference in AMACR expression in the 37 naive primary prostate samples and lymph node metastases derived from the same patient (Mann Whitney test, $p=0.8$). In other words, matched primaries and lymph node metastases showed similar AMACR expression pattern.

A subset of 22 PCA cases in which the patients received variable amount and types of anti-androgen treatment prior to surgery was examined. These cases were evaluated blindly with respect to treatment protocol for histological evidence of hormone treatment (H&E slide) and AMACR protein expression. The hormonal effect visible on the H&E slides was classified from 1 to 4 with 1 representing "no effect" and 4 showing

a "very strong effect". 13 cases demonstrated either no or moderate hormonal effect, and 9 cases had a very strong hormonal effect. Statistical analysis revealed a significant difference between these two groups with respect to AMACR expression intensity (Figure 17, Mantel-Haenszel Chi-Square, p=0.009). Figure 17 presents an example of a PCA case treated prior to surgery with anti-androgens that has a strong hormonal effect appreciated on H&E and decreased AMACR protein expression (Figure 17A). In this dataset there was neither a correlation between treatment duration nor treatment type (monotherapy or complete hormonal withdrawal for hormone deprivation) and AMACR expression.

For further exploration of the hormonal effect on AMACR expression, primary cell culture experiments and Western blot analysis were performed. As demonstrated in Figure 17 Panel B, LNCaP cells, derived from a metastatic lesion but considered hormone responsive, showed a higher baseline AMACR expression as compared to PC3 and DU-145 cells, which are both hormone independent cell lines derived from metastatic lesions. A benign cell line, RWPE-1 (Bello *et al.*, Carcinogenesis 18:1215 [1997]), showed near absent AMACR expression, which is consistent with the in situ protein expression data. To simulate an anti-androgen treatment, the hormone responsive cell line LNCaP was treated with bicalutamide in a final concentration of 20 μM for a time period of 24 and 48 hours. AMACR expression in cell lysates of LNCaP cells did not change at either time point when exposed to anti-androgen therapy. Under the same conditions, PSA, a gene known to be regulated by the androgen receptor, showed decreased protein expression. In addition, when LNCaP cells were exposed to a synthetic androgen R1881, no increase in AMACR expression was observed (Figure 17, Panel B). Therefore, these cell culture experiments provide evidence that AMACR expression is not regulated by the androgen pathway.

The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that another explanation for these observations was that AMACR over expression occurred in PCA, but as these tumors became poorly differentiated, as in the hormone refractory PCA, AMACR expression was down regulated either directly or indirectly due to the process of de-differentiation. To

elucidate this potential correlation colon cancer samples were examined for AMACR expression (See Example 7). AMACR protein expression is also observed in some other tumor types, with the highest overall expression in colorectal cancers. Colorectal cancers are not known to be regulated by androgens and were therefore used as a control to test this hypothesis. Four well differentiated and seven anaplastic colon cancer samples were chosen. The poorly differentiated tumors have distinct molecular alterations distinguishing them from the common well to moderately differentiated colorectal tumors (Hinoi *et al.*, Am. J. Pathol. 159:2239 [2001]). Strong AMACR protein expression in a moderately differentiated colon cancer was observed. This tumor still forms well defined glandular structures. The surrounding benign colonic tissue does not express AMACR. The anaplastic colon cancers demonstrated weak AMACR protein expression. Primarily data revealed positive AMACR expression in 4/4 well differentiated cases but only 4/7 anaplastic colonic cancers. Three of the anaplastic colon cancers had weak to moderate expression. Metastatic hormone refractory PCA demonstrated weak AMACR protein expression in tissue microarrays.

Example 7

AMACR Expression in a Variety of Cancers

A. Analysis of online EST and SAGE database

The National Cancer Institute Cancer Genome Anatomy Project (CGAP) has several gene expression databases available online for comparing gene expression across multiple samples (See the Internet Web site of the National Cancer Institute). Both EST and SAGE databases offer Virtual Northern blots, which allow users to visualize and compare the expression level of a particular gene among multiple samples. The SAGE database includes over 5 million tags from 112 libraries of multiple benign and malignant tissues.

B. Selection of study cases

A total of 96 cases of cancers from different sites were selected for construction of a multi-tumor tissue microarray. The tissue microarray was constructed to perform a

wide survey of multiple common tumor types. A minimum of three tissue cores (0.6 mm in diameter) was taken for each case. Tumors surveyed included colorectal adenocarcinoma (n=15 cases), renal cell carcinoma (6), prostatic adenocarcinoma (6), urothelial carcinoma (4), cervical squamous cell carcinoma (6), lung non-small cell carcinoma (4), lymphoma (15), melanoma (9) and several other cancer types. Normal adjacent tissue was taken when available. The prostate tissue microarray was constructed from selected patients who underwent radical prostatectomies as monotherapy for clinically localized prostate cancer. This tissue microarray contained a spectrum of prostatic tissue including prostatic atrophy, high-grade prostatic intraepithelial neoplasia (PIN), and clinically localized prostate cancer. In addition, standard slides were used to confirm results for colon cancer. Twenty-four cases of colorectal adenocarcinoma (16 well to moderately differentiated carcinoma and 8 large cell minimally differentiated carcinoma) and 8 endoscopically derived colorectal adenomas were selected for immunostaining for AMACR. For breast carcinoma, a TMA of 52 cases of invasive ductal carcinoma was used. Specimens were collected and analyzed in accordance with the Institutional Review Board guidelines.

C. Immunohistochemistry

Standard avidin-biotin complex immunohistochemistry was used. Pre-treatment was performed by steaming the slides for 10 minutes in sodium citrate buffer in a microwave oven. The slides were then incubated sequentially with primary antibody (1:2000 dilution, polyclonal rabbit anti-AMACR antibody), biotinylated secondary antibody, avidin-biotin complex and chromogenic substrate 3,3'-diaminobenzidine. Slides were evaluated for adequacy using a standard bright field microscope. Digital images were then acquired using the BLISS Imaging System (Bacus Lab, Lombard, IL) and evaluated by two pathologists. Protein expression was scored as negative, weak stain (faint cytoplasmic stain or granular apical staining), moderate (diffuse granular cytoplasmic stain) and strong (diffuse intense cytoplasmic stain). Only moderate and strong staining was considered as positive staining.

D. Laser Capture Microdissection

Sections of 2 radical prostatectomy samples were frozen in OCT in accordance with an Institutional Review Board protocol. Frozen sections (5 μ m thick) were fixed in 70% alcohol for 10 minutes and then stained in hemotoxylin and eosin. Prostate cancer and benign prostate glands were dissected on a μCUT laser capture microdissector (MMI GmbH, Heidelberg, Germany). Approximately 6000 cells were harvested. Total RNA was isolated using Qiagen micro-isolation kit (Qiagen, San Diego, CA). Reverse transcription was performed using both oligo dT and random hexamer primers. Primers used to amplify specific gene products were: AMACR sense, 5'-CGTATGCCCCGCTGAATCTCGTG-3' (SEQ ID NO:104); AMACR antisense, 5'-TGGCCAATCATCCGTGCTCATCTG-3' (SEQ ID NO:105); GAPDH sense, 5'AGCCTTCTCCATGGTGGTGAAGAC-3' (SEQ ID NO:106); and GAPDH antisense, 5'-AGCCTTCTCCATGGTGGTGAAGAC-3' (SEQ ID NO:107). PCR conditions for AMACR and GAPDH were: heat denaturation at 94°C for 5 min, cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min (32 cycles for GAPDH, 40 cycles for AMACR), and a final extension step at 72°C for 5 min. PCR products were then separated on 2% agarose gel and visualized by UV illumination.

E. Results

Using the Virtual Northern tool from the online CGAP program, AMACR expression was surveyed in two databases, EST and SAGE libraries. AMACR was found to be expressed in a wide range of tissues, including central and peripheral nervous system, colon, kidney, breast, pancreas, prostate and blood. Compared to their normal counterparts, a number of cancers have elevated AMACR expression, including tumors arising in bone marrow, breast, colon, genitourinary system, lung, lymph node, nervous system, pancreas, prostate, soft tissue and uterus.

To confirm the gene expression data, AMACR immunohistochemistry was performed on a multi-tumor tissue array that included some of the most common cancers from multiple sites. AMACR protein level was increased in many cancers, including colorectal, prostate, ovarian, lung cancers, lymphoma and melanoma (Figure 18). In particular, AMACR over-expression was observed in 92% and 83% of colorectal and

prostate cancer, respectively. Using a breast cancer tissue microarray, it was found that AMACR over-expression was present in 44% of invasive ductal carcinomas. AMACR over expression was not observed in female cervical squamous cell carcinoma (6 cases).

To further characterize AMACR expression in a spectrum of proliferative prostate lesions, a prostate tissue microarray, which included prostate cancer, high grade PIN and atrophic glands, was utilized. Positive AMACR staining (moderate and strong staining) was observed in 83% and 64% of clinically localized prostate cancer and high-grade PIN, respectively. Focal AMACR expression was observed in 36% of the atrophic lesions and in rare morphologically benign glands. To confirm that AMACR protein over-expression was the result of increased gene transcription, laser capture microdissection was used to isolate cancerous and benign prostatic glands. RT-PCR was performed to assess the AMACR mRNA expression. Benign glands had very low baseline expression (Figure 19). In contrast, prostate cancer had much higher mRNA level, confirming that increased AMACR gene transcription leads to elevated protein over expression in prostate cancer.

AMACR expression was studied in 24 colorectal adenocarcinomas, including 16 well to moderately differentiated, and 8 poorly differentiated large cell adenocarcinomas. Overall, 83% (20/24) demonstrated positive AMACR protein expression. All (16/16, 100%) cases of well to moderately differentiated carcinoma had positive staining, compared to 64% (5/8) of poorly differentiated carcinoma. AMACR expression was examined in 8 colorectal adenoma biopsies obtained by colonoscopy. Moderate staining was present in 6 (75%) cases. Compared with well-differentiated adenocarcinomas, adenomas usually showed more focal (10-60% of cells) and less intense staining.

Example 8

Characterization of EZH2 expression in Prostate Cancer

A. SAM Analysis

SAM analysis was performed by comparing gene expression profiles of 7 metastatic prostate cancer samples against 10 clinically localized prostate cancer samples. Data was normalized per array by multiplication by a factor to adjust the aggregate ratio of medians to one, then log base 2 transformed and median centered. This normalized

data was divided into two groups for comparison using a two-class, unpaired t-test. Critical values for the analysis include: Iterations = 500, Random Number Seed 1234567, a fold change cutoff of 1.5 and a delta cutoff of 0.985, resulting in a final largest median False Discovery Rate of 0.898 % for the 535 genes selected as significant (55 relatively up and 480 relatively down regulated between MET and PCA). These 535 genes were analyzed using Cluster (Eisen *et al.*, PNAS 95:14863 [1998]) implementing average linkage hierarchical clustering of genes. The output was visualized by Treeview (Eisen *et al.*, [1998], *supra*).

B. RT-PCR

Reverse transcription and PCR amplification were performed with 1 μg total RNA isolated from the indicated prostate tissues and cell lines. Human EZH2 forward (5'-GCCAGACTGGGAAGAAATCTG-3' (SEQ ID NO:108)), reverse (5'-TGTGCTGGAAAATCCAAGTCA-3' (SEQ ID NO:109)) and GAPDH sense (5'-CGGAGTCAACGGATTTGGTCGTAT- 3' (SEQ ID NO:110)), antisense 5'-AGCCTTCTCCATGGTGGTGAAGAC-3' (SEQ ID NO:111)) primers were used. The amplified DNA was resolved on agarose gels and visualized with ethidium bromide.

C. Immunoblot Analysis

Prostate tissue extracts were separated by SDS-PAGE and blotted onto nitrocellulose membranes. Anti-EZH2 (Sewalt *et al.*, Mol. Cell. Biol. 18:3586 [1998]), anti-EED (Sewalt *et al.*, *supra*), and polyclonal anti- tubulin (Santa Cruz biotechnology) antibodies were used at 1:1000 dilution for immunoblot analysis. The primary antibodies were detected using horseradish peroxidase-conjugated secondary antibodies and visualized by enhanced chemiluminescence as described by the manufacturer (Amersham-Pharmacia).

D. Tissue Microarray Analysis

Clinically stratified prostate cancer tissue microarrays used in this study have been described previously (See above examples). Tissues utilized were from the radical prostatectomy series at the University of Michigan and from the Rapid Autopsy Program,

which are both part of University of Michigan Prostate Cancer Specialized Program of Research Excellence (S.P.O.R.E.) Tissue Core. Institutional Review Board approval was obtained to procure and analyze the tissues used in this study.

EZH-2 protein expression was evaluated on a wide range of prostate tissue to determine the intensity and extent *in situ*. Immunohistochemistry was performed on three tissue microarrays (TMA) containing samples of benign prostate, prostatic atrophy, high-grade prostatic intraepithelial neoplasia (PIN), clinically localized prostate cancer (PCA), and metastatic hormone refractory prostate cancer (HR-METSs). Standard biotin-avidin complex immunohistochemistry (IHC) was performed to evaluate EZH2 protein expression using a polyclonal anti-EZH2 antibody. Protein expression was scored as negative (score=1), weak (score 2), moderate (3) and strong (4).

Approximately 700 TMA samples (0.6 mm diameter) were evaluated for this study (3-4 tissue cores per case). The TMAs were assembled using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD) as previously described (See above examples). Four replicate tissue cores were sampled from each of the selected tissue types. After construction, 4 μm sections were cut and hematoxylin and eosin staining was performed on the initial slide to verify the histologic diagnosis. TMA hematoxylin and eosin images were acquired using the BLISS Imaging System (Bacus Lab, Lombard, IL). EZH2 protein expression was evaluated in a blinded manner by the study pathologist using a validated web-based tool (Manley *et al.*, Am. J. Pathol. 159:837 [2001]; Bova *et al.*, Hum. Pathol. 32:417 [2001]) and the median value of all measurements from a single patient were used for subsequent analysis.

E. Clinical Outcomes Analysis

To assess individual variables for risk of recurrence, Kaplan-Meier survival analysis was performed and a univariate Cox proportional hazards model was generated. PSA-recurrence was defined as 0.2 ng/ml following radical prostatectomy. Covariates included Gleason sum, preoperative PSA, maximum tumor dimension, tumor stage, and surgical margin status. To assess the influence of several variables simultaneously including EZH2 protein expression, a final multivariate Cox proportional hazards model of statistically significant covariates was generated. Statistical significance in univariate

and multivariate Cox models were determined by Wald's test. A p-value <0.05 was considered statistically significant.

F. EZH2 Constructs

Myc-tagged EZH2-pCMV was used. The Myc-EZH2 fragment was released with BamHI/XhoI double digest and was sub-cloned into the mammalian expression vector pCDNA3 (Invitrogen). An EZH2-ER in-frame fusion expression construct was generated by replacing the *FADD* fragment released by Kpn I/Not I double digest of the FADD-ER construct (originally derived from Myc-ER (Littlewood *et al.*, Nuc. Acids. Res. 23:1686 [1995]) with the PCR amplified human EZH2 devoid of its stop codon. The EZH2 .SET mutant DNA was amplified using the primers 5'G*GGGTAC*CATGGGCGGCCGCGAACAAAAGTTGATT 3' (SEQ ID NO:112) and 5'GGGGAATTCTCATGCCAGCAATAGATGCTTTTT3' (SEQ ID NO:113) and subsequently sub-cloned into pCDNA3 utilizing the in built KpnI/EcoRI sites. Expression of these constructs was verified by immunoblot analysis of the expressed proteins using either anti-Myc HRP (Roche, Inc) or anti-EZH2 antibodies.

G. RNA interference

21-nucleotide sense and antisense RNA oligonucleotides were chemically synthesized (Dharmacon Research Inc.) and annealed to form duplexes. The siRNA employed in the study were targeted to the region corresponding from 85 to 106 of the reported human EZH2 (NM004456). Control siRNA duplexes targeted luciferase, lamin and AMACR (NM014324). The human transformed prostate cell line RWPE (Webber *et al.*, Carcinogenesis 18:1225 [1997]) and PC3 were plated at $2x10^5$ cells per well in a 12 well plate (for immunoblot analysis, cell counts, and fluorescence activated cell sorting (FACS) analysis) and $1.5x10^4$ cell per well in a 96 well plate (for WST-1 proliferation assays). Twelve hours after plating, the cells were transfected with 60 picomoles of siRNA duplex, sense or antisense oligonucleotides (targeting EZH2) using oligofectamine (Invitrogen). A second identical transfection was performed 24 hours later. Forty-eight hours after the first transfection, the cells were lysed for immunoblot

analysis and trypsinized for cell number estimation or FACS analysis. Cell viability was assessed 60 hours after the initial transfection.

H. Cell Proliferation Assays

Cell proliferation was determined with the colorimetric assay of cell viability, based on the cleavage of tetrazolium salt WST-1 by mitochondrial dehydrogenases as per manufacturers instructions (Roche, Inc.). The absorbance of the formazan dye formed, which directly correlates with the number of metabolically active cells in the culture, was measured at 450nm (Bio-Tek instruments), an hour after the addition of the reagent. Cell counts were estimated by trypsinizing cells and analysis by coulter cell counter.

I. Flow cytometric analysis

Trypsinized cells were washed with phosphate buffered saline (PBS) and cell number was determined by using a coulter cell counter. For FACS analysis, the washed cells were fixed in 70% ethanol overnight. Before staining with propidium iodide, the cells were washed again with PBS and analyzed by flow cytometry (Becton Dickinson).

J. Microarray analysis of EZH2 transfected cells

Initial testing of this transient transfection/transcriptome analysis system demonstrated that transient overexpression of TNFR1 (p55), a receptor for tumor necrosis factor, induced similar expression profiles as was observed with incubation of cells with TNF (Kumar-Smith *et al.*, J. Biol. Chem. 24:24 [2001]). Other molecules have been similarly tested with this approach. Cells were transfected with different EZH2 constructs and transfection efficiency was monitored by beta-galactosidase assay and was approximately 30-50%. EZH2 .SET mutant expressing samples were compared to EZH2 expressing samples using the SAM analysis package (Tusher *et al.*, PNAS 98:5116 [2001]). Data was pre-processed by multiplication by a normalization factor to adjust the aggregate ratio of medians to one, log base 2 transformed and median centered each array, individually. This pre-processed data was divided into 2 groups for comparison using a two-class, unpaired t-test. Critical values for the analysis include: iterations = 5000, (720 at convergence) random Number Seed 1234567, a fold change of 1.5 and a

delta cutoff of 0.45205, resulting in a final largest median False Discovery Rate of 0.45% for the 161 genes selected as significant. These 161 genes were supplemented by the values for EZH2 and then analyzed using Cluster implementing average linkage hierarchical clustering of genes. The output was visualized in Treeview. Selected genes identified as being repressed by EZH2 (*e.g.*, EPC and cdc27) were re-sequenced to confirm identity.

The molecular identity of a cell is determined by the genes it expresses (and represses). Embryogenesis and cell differentiation intimately depend upon keeping certain genes "on" and other genes "off". When the transcriptional "memory" of a cell is perturbed this can lead to severe developmental defects (Jacobs *et al.*, Semin. Cell Dev. Biol. 10:227 [1999]; Francis *et al.*, Nat. Rev. Mol. Cell. Biol. 2:409 [2001]). Lack of differentiation, or anaplasia, is a hallmark of cancer, which results from normal cells "forgetting" their cellular identity. Thus, it is not surprising that dysregulation of the transcriptional maintenance system can lead to malignancy (Francis *et al.*, *supra*; Jabobs *et al.*, Nature 397:164 [1999]; Beuchle *et al.*, Development 128:993 [2001]).

Studies in *Drosophila melanogaster* have been instrumental in the understanding of the proteins involved in transcriptional maintenance (Beuchle *et al.*, [[2001], *supra*; Strutt *et al.*, Mol. Cell. Biol. 17:6773 [1997]; Tie *et al.*, Development 128:275 [2001]). Two groups of proteins have been implicated in the maintenance of homeotic gene expression and include polycomb (PcG) and trithorax (trxG) group proteins (Mahmoudi *et al.*, Oncogene 20:3055 [2001]; Lajeunesse *et al.*, Development 122:2189 [1996]). PcG proteins act in large complexes and are thought to repress gene expression, while trxG proteins are operationally defined as antagonists of PcG proteins and thus activate gene expression (Francis *et al.*, Nat. Rev. Mol. Cell. Biol. 2:409 [2001]; Mahmoudi *et al.*, *supra*). There are at least twenty PcG and trxG proteins in *Drosophila*, and many have mammalian counterparts. In human malignancies, PcG and trxG proteins have primarily been found to be dysregulated in cells of hematopoietic origin (Yu *et al.*, Nature 378:505 [1995]; Raaphorst *et al.*, Am. J. Pathol., 157:709 [2000]; van Lohuizzen *et al.*, Cell 65:737 [1991]). EZH2 is the human homolog of the *Drosophila* protein Enhancer of Zeste (E(z)) ((Laible *et al.*, Embo. J. 16:3219 [1997]), for which genetic data defines as a PcG protein with additional trxG properties (LaJeunesse *et al.*, *supra*). E(z) and EZH2

share homology in four regions including domain I, domain II, a cysteine-rich amino acid stretch, and a C-terminal SET domain (Laible *et al.*, *supra*). The SET domain is a highly conserved domain found in chromatin-associated regulators of gene expression often modulating cell growth pathways (Jenuwein *et al.*, Cell. Mol. Life Sci. 54:80 [1998]). EZH2 is thought to function in a PcG protein complex made up of EED, YY1 and HDAC2 (Satijn *et al.*, Biochim. Biophys. Acta. 1447:1 [1999]). Disruption of the EZH2 gene in mice causes embryonic lethality suggesting a crucial role in development (O'Carroll *et al.*, Mol. Cell. Biol. 21:4330 [2001]).

In previous studies (*See e.g.*, Example 1), the gene at the top of the "list" of genes significantly up-regulated in metastatic prostate cancer was EZH2, which had a d-score (Tusher *et al.* PNAS 98:5116 [2001]) of 4.58 and a gene-specific FDR of 0.0012 (also called a "q-value" which is analogous to p-values, but adapted to multiple inference scenarios. Figure 20a displays the 55 up-regulated genes identified by this approach. Figure 20b summarizes the gene expression of EZH2 in 74 prostate tissue specimens analyzed on DNA microarrays made up of 10 K elements. The EZH2 transcript was significantly increased in metastatic prostate cancer with respect to clinically localized prostate cancer (Mann-Whitney test, p=0.001) and benign prostate (p=0.0001).

As independent experimental validation of DNA microarray results, RT-PCR was performed on 18 prostate samples and cell lines. As expected, EZH2 mRNA transcript levels were elevated in malignant prostate samples relative to benign (Fig. 20c).
To determine whether EZH2 is up-regulated at the protein level in metastatic prostate cancer, tissue extracts were examined by immunoblotting. In the samples examined by immunoblot analysis, EZH2 protein was markedly elevated in metastatic prostate cancer relative to localized prostate cancer or benign prostate (Fig. 20d).

Importantly, EED, a PcG protein that forms a complex with EZH2 (vanLohuizen *et al.*, *supra*; Sewalt *et al.*, *supra*), along with an un-related protein, ß-tubulin, did not exhibit similar protein dysregulation. EZH2 protein expression was evaluated on a wide range of prostate tissues (over 700 tissue microarray elements) to determine the intensity and extent of expression *in situ* (Fig. 21 a,b). When highly expressed, EZH2 expression was primarily observed in the nucleus as suggested previously (Raaphorst *et al.*, *supra*). The staining intensity was increased from benign, prostatic atrophy, prostatic

intraepithelial neoplasia (PIN), to clinically localized prostate cancer with median staining intensity of 1.7 (standard error [SE], 0.1; 95% confidence interval [CI], 1.5-1.9), 1.7 (SE, 0.2; 95%CI, 1.3-2.0), 2.3 (SE, 0.2.; 95%CI, 1.9-2.7), and 2.6 (SE, 0.1; 95%CI, 2.4-2.8), respectively (Fig. 24b). The strongest EZH2 protein expression was observed in hormone-refractory metastatic prostate cancer with a median staining intensity of 3.3 (SE, 0.3; 95%CI, 2.7-3.9). There was a statistically significant difference in EZH2 staining intensity between benign prostate tissue and localized prostate cancer (ANOVA post-hoc analysis mean difference 0.9, $p<0.0001$). Although metastatic prostate cancer had a higher mean expression level than localized prostate cancer, the difference did not reach statistical significance (ANOVA post-hoc analysis mean difference 0.7, $p=0.3$). These findings suggest that as prostate neoplasia progresses there was a trend towards increased EZH2 protein expression, mimicking that seen by DNA expression array analysis. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that this observation suggests that EZH2 levels may indicate how aggressive an individual's prostate cancer is given that the highest level of expression was observed in hormone-refractory, metastatic prostate cancer. Therefore, to test this hypothesis, the utility of EZH2 protein levels to predict clinical outcome in men treated with surgery for clinically localized prostate cancer was examined.

Two hundred and twenty-five (225) specimens from sixty-four patients (3-4 replicate samples per patient) with clinical follow up were interrogated on a single tissue microarray. These men had a median age of 61 years (range 43-76 years) and a 7.3 ng/ml median pre-operative serum prostate specific antigen (PSA) (range 0.8-21.0 ng/ml). Pathologic examination of their prostatectomy specimens indicated that 77% had organ-confined disease (pT2 stage) and 72% had negative surgical margins. The patient demographics and tumor stages were representative of the over 1500 radical prostatectomy patients. In order to test the utility of EZH2 as a potential tissue biomarker for prostate cancer, the clinical outcome of these 64 cases was examined, taking into account clinical and pathological parameters. Clinical failure was defined as either a 0.2 ng/ml PSA elevation or disease recurrence following prostatectomy (*e.g.*, development of metastatic disease). By Kaplan-Meier analysis (Fig. 21c), EZH2 staining intensity of 3

and greater was significantly associated with clinical failure in 31% (10/32) of patients in contrast to 9% (3/32) of patients with an EZH2 protein levels below 3 (log rank p=0.03). There was no significant correlation between EZH2 levels and Gleason score (<7 versus =7), tumor stage (pT2 versus pT3), or surgical margin status (negative versus positive). There was a significant (p=0.048) albeit weak (Pearson coefficient =0.33) correlation between EZH2 protein levels and proliferation index *in situ* as assessed by Ki-67 labeling index. Multivariable Cox-Hazards regression analysis revealed that EZH2 protein expression (=3 versus <3) was the best predictor of clinical outcome with a recurrence ratio of 4.6 (95%CI 1.2-17.1, p=0.02), which was significantly better than surgical margin status, maximum tumor dimension, Gleason score, and pre-operative PSA. Thus, monitoring EZH2 protein levels in prostate specimens may provide additional prognostic information not discernible with current clinical and pathology parameters alone.

To shed light into the functional role of EZH2 in prostate cancer progression, EZH2 expression in transformed prostate cells *in vitro* was disrupted using RNA interference. T. Tuschl and colleagues recently reported that duplexes of 21-nucleotide RNA (siRNAs) mediate RNA interference in cultured mammalian cells in a gene-specific fashion (Elbashir *et al.*, Nature 411:494 [2001]). RNA interference has been used effectively in insect cell lines to "knock-down" the expression of specific proteins, owing to sequence-specific, double stranded-RNA mediated RNA degradation (Hammond *et al.*, Nature 404:293 [2000]). siRNAs are potent mediators of gene silencing, several orders of magnitude more potent than conventional antisense or ribozyme approaches (Macejak *et al.*, Hepatology 31:769 [2000]). Thus, a 21- nucleotide stretch of the EZH2 molecule was targeted using criteria provided by Elbashir *et al.* (*supra*), and RNA oligonucleotides were synthesized commercially. After the RNA oligos were annealed to form siRNA duplexes, they were tested on the transformed androgen-responsive prostate cell line RWPE (Webber *et al.*, Carcinogenesis 18:1225 [1997]; Bello *et al.*, Carcinogenesis 18:1215 [1997]) as well as the metastatic prostate cancer cell line PC3. Forty-eight hours after transfection with siRNA duplexes, the levels of endogenous EZH2 protein were quntitated. When EZH2 protein was specifically down-regulated in prostate cell lines, the levels of the un-related control protein, β-tubulin, remained unchanged (Fig. 22a). The sense or anti-sense oligonucleotides comprising the EZH2 duplex, as well as un-

related siRNA duplexes, did not affect EZH2 protein levels (Fig. 22a, middle and right panels), verifying the specificity of the siRNA approach in both prostate cell lines.

The phenotype of EZH2 "knock-down" prostate cells was next examined. By phase contrast microscopy, it was observed that siRNA directed against EZH2 markedly inhibited cell number/confluency relative to buffer control. Cell counts taken 48 hrs after transfection with siRNA showed a 62% inhibition of RWPE cell growth mediated by the EZH2 siRNA duplex, which is in contrast to the corresponding sense and anti-sense EZH2 oligonucleotides or control duplexes (targeting luciferase and lamin) which exhibited minimal inhibition (Fig. 22b). The prostate cancer cell line, PC3, demonstrated a similar growth inhibition mediated by EZH2 siRNA, suggesting that the findings are not a peculiarity of the RWPE cell line (Fig. 22b). Using a commercially available cell proliferation reagent WST-1, which measures mitochondrial dehydrogenase activity, a decrease in cell proliferation mediated by the EZH2 siRNA duplex, but not by un-related duplexes, was observed (Fig. 22c). In the time frame considered (48hrs), RNA interference of EZH2 did not induce apoptosis as assessed by propidium idodide staining of nuclei or PARP cleavage. Consistent with this, the broad-spectrum caspase inhibitor, z-VAD-fmk, failed to attenuate EZH2 siRNA induced inhibition of cell proliferation (Fig. 22c). Thus, activation of the apoptosis pathway does not account for the decreases in cell number observed by RNA interference of EZH2.

Various PcG Group proteins have been suggested to play a role in cell cycle progression (Jacobs *et al.*, Nature 397:164 [1999]; Visser *et al.*, Br. J. Hematol. 112:950 [2001]; Borck *et al.* Curr. Opin. Genet. Dev. 11:175 [2001]). Flow cytometric analysis of EZH2 siRNA-treated prostate cells demonstrated cell cycle arrest in the G2/M phase (Fig. 22d). Un-related control siRNA duplexes failed to induce a similar cell cycle dysregulation. Few apoptotic cells (sub-G1 cells) were present in any of the experimental samples tested as assessed by flow cytometry (Fig. 22d). The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that these observations suggest that EZH2 plays a role in prostate cell proliferation by mitigating the G2/M transition.

To further understand the functional role of EZH2 in prostate cells, an epitope-tagged version of wild-type EZH2 and a deletion mutant of EZH2 missing the conserved SET domain in the eukaryotic expression vector pcDNA3 were generated (Fig. 23a). An "inducible"-version of EZH2 was also generated by creating a fusion protein with a modified murine estrogen receptor (ER) (Fig. 26a) (Littlewood *et al.*, Nuc. Acid. Res. 23:1686 [1995]; Juin *et al.*, Genes Dev. 13:1367 [1999]). EZH2-ER fusion was expressed in cells (Fig. 26b) and is inactivated, presumably by sequestration/binding to hsp90 and other proteins (Littlewood *et al.*, *supra*). Upon treatment of cells with 4-hydroxytamoxifen, hsp90 dissociates from the ER fusion and liberates its activity. Expression of the epitope-tagged EZH2 constructs was confirmed by transfection in 293 (Fig. 23b), RWPE and in other mammalian cell lines.

PcG proteins have been proposed to mediate their functions by repression of target genes (Laible *et al.*, *supra*; Jacobs *et al.*, Semin Cell Dev. Biol. 10:227 [1999]). To begin to test this hypothesis, RWPE prostate cells were transiently transfected with wild-type EZH2 and global gene expression alterations were monitored using DNA microarrays. While RNA from the experimental (transfected) cell line was labeled with one fluorescent dye, the paired reference sample was labeled with a second distinguishable fluorescent dye. By making direct comparisons between "gene"-transfected cell lines and control vector-transfected cell lines the molecular differences between the samples were observed. When EZH2 was over-expressed in RWPE cells or SUM149 breast carcinoma cells, there was a consistent repression of a cohort of genes (Fig. 23c, d). This exclusive repression of genes was unique compared to other molecules tested in this system including c-myc and TNFR1, among others. When compared to vector-transfected cells the only gene that was significantly up-regulated in EZH2-transfected cells was EZH2 itself (Fig. 23c).

EZH2-mediated transcriptional repression was dependent on an intact SET domain (Fig. 23c), as deletion of this domain did not produce a repressive phenotype and in some cases "de-repressed" genes. EZH2 has been shown to interact with histone deacetylase 2 (HDAC2) via the EED protein (van der Vlag *et al.*, Nat. Genet. 23:474 [1999]). In the experiments described above, EZH2-mediated gene silencing was dependent on HDAC activity, as the commonly used HDAC inhibitor, trichostatin A

(TSA) completely abrogated the effects of EZH2 (Fig. 23c). Thus, EZH2 function requires both an intact SET domain as well as endogenous HDAC activity.

To identify genes that are significantly repressed by EZH2, wild-type EZH2-transfected cells were compared with EZH2 .SET-transfected cells. Using this approach, 163 genes were consistently repressed while no genes were activated at an FDR of 0.0045 (Fig. 23d). Examination of the significant gene list identified the PcG group protein EPC, which is the human homolog of the *drosophila* protein Enhancer of Polycomb (E(Pc)) as being consistently repressed by EZH2 (Fig. 23c). Of the *Drosophila* PcG proteins, E(Pc) and E(z) are related in that they both act as suppressors of variegation (Su(var)) (Sinclair *et al.*, Genetics 148:211 [1998]) and are the only PcG proteins to have yeast homologs, emphasizing the evolutionary conservation of this PcG pair. In addition to EPC, a host of other transcriptional regulators/activators were transcriptionally silenced by EZH2 including MDNA, RNF5, RNF15, ZNF42, ZNF262, ZNFN1A1, RBM5, SPIB, and FOXF2, among others (Fig. 23c). MDNA, also known as myeloid cell nuclear differentiation antigen, mediates transcriptional repression by interacting with the transcription factor YY1, which is a PcG homolog of *Drosophila* Pho and shown to be part of the EZH2/EED complex of proteins (Satijin *et al.*, Mol. Cell. Biol. 21:1360 [2001]).

In addition to transcriptional repression in prostate cells, the results also support a role for EZH2 in regulating cell growth (Fig. 23). Transcriptional repression of cdc27 (two independent Unigene clones) was also observed. Cdc27 is part of the anaphase-promoting complex (APC) which mediates ubiquitination of cyclin B1, resulting in cyclinB/cdk complex degradation (Jorgensen *et al.*, Mol. Cell. Biol. 18:468 [1998]). Another family of proteins that was repressed when EZH2 was targeted was the solute carriers. At least 5 distinct members were shown to be repressed (*i.e.*, SSLC34A2, SLC25A16, SLC25A6, SLC16A2, and SLC4A3).

Example 9

Expression of AMACR in Serum and Urine

This example describes the expression of AMACR in serum and urine. AMACR was detected by standard immunoblotting and by protein microarray using a polyclonal

rabbit anti-AMACR antibody. The results are shown in Figures 24-27. Figure 24 shows the detection of AMACR protein in PCA cell lines by quantitation of microarray data. DUCAP, DU145, and VCAP are prostate cancer cell lines. RWPE is a benign prostate cell line. PHINX is a human embryonic kidney cell line.

Figure 25 shows the detection of AMACR protein in serum by quantitation of microarray data. P1-P7 represent serum from patients with prostate cancer. NS2 and NS3 represent serum from patients that do not have PCA. SNS2 and SNS3 represent serum from patients that do not have PCA that has been spiked with AMACR protein. Figure 26 shows an immunoblot analysis of serum from patients with either negative or positive PSA antigen. Figure 27 shows an immunoblot analysis of the presence of AMACR in urine samples from patients with bladder cancer (females) or bladder cancer and incidental prostate cancer (males). The results demonstrate that AMACR can be detected in the serum and urine of patients with bladder cancer or bladder cancer and prostate cancer.

Example 10

AMACR as a Tumor Antigen

This example describes the presence of an immune response against AMACR in serum. Figure 28 shows representative data of a humoral response by protein microarray analysis. Tumor antigens including AMACR, PSA, CEA, HSPs were spotted onto nitrocellulose coated slides. The slides were incubated with sera from different patients to detect a humoral response. The microarray was then washed. A Cy5 labeled goat anti-human IgG was used to detect the humoral response. The slide was then scanned using a microarray scanner (Axon). After data normalization, intensity of spots reflects the presence, absence or strength of humoral response to specific tumor antigen. A specific humoral response to AMACR was detected in cancer patients but not in controls. Cancer refers to sera from prostate cancer patients. BPH refers to sera from patients with benign prostate hyperplasia.

Figure 29 shows immunoblot analysis of the humoral response to AMACR. Figure 29A shows an SDS-PAGE gel containing recombinant MBP (control protein=M) and recombinant AMACR-MBP (A) that was run and transferred to nitrocellulose paper.

Each strip blot was then incubated with human sera. A humoral response to the AMACR was detected using an HRP-conjugated anti-human antibody. Only AMACR and fragments of AMACR were detected in sera from prostate cancer patients and not in controls. Figure 29B shows a control experiment whereby the humoral response is blocked with recombinant AMACR (quenched) and thus shows the specificity of the response.

This example demonstrates that AMACR functions as a tumor antigen in human serum of prostate cancer patients. A specific immune response was generated to AMACR in the serum of PCA patients, but not in controls.

Example 11

Expression of GP73 in Prostate Cancer

This example describes the association of GP73 with prostate cancer.

A. Methods

Microarray analysis, RT-PCR, Western blotting, and immunohistochemistry were performed as described in the above examples.

B. Results

Figure 30 shows GP73 Transcript levels in prostate cancer. Figure 30a shows the level of GP73 in individual samples after microarray analysis. The graph shows the values of Cy5 versus Cy3 ratio wherein the prostate cancer tissue sample RNA were labeled with Cy5 fluorescent dye, while the reference sample (pool of benign tissue RNA) sample was labeled with Cy3 fluorescent dye. A total of 76 individual experiments from different prostate tissue are plotted and they are classified as benign, prostate cancer and metastatic cancer types. Figure 30b shows the result of GP73 transcripts determined by DNA microarray analysis from 76 prostate samples grouped according to sample type and averaged. The experimental samples were labeled with Cy5 fluorescent dye, whereas the reference sample (pool of benign tissue sample) was labeled with Cy3 fluorescent dye. The box plot demonstrates the range of GP73 expression within each group. The middle horizontal bar indicates median values; the

upper and lower limits of the boxes, interquartile ranges; and the error bars, 95% confidence intervals. Figure 30c demonstrates that GP73 transcript levels are elevated in prostate cancer. RT-PCR was used to detect GP73 transcript levels in RNA preparations from prostate tissue extracts. GAPDH served as the loading control.

Figure 31 shows that GP73 protein is upregulated in prostate cancer. Figure 31a shows Western blot analysis of GP73 protein in prostate cancer. Total tissue proteins from benign, cancer and metastatic tissues (10 μg) were analyzed using anti-GP73 antiserum. β-Tubulin serves as control for sample loading. Figure 31b shows an immunoblot analysis of the Golgi resident protein Golgin 97. The Golgin 97 protein levels were analyzed in the prostate tissue sample to indicate the level of Golgi structure in normal and cancerous prostate tissue. β-Tubulin serves as control for sample loading.

Tissue microarray analysis of GP73 protein in normal and cancerous prostate tissue was also performed. GP73 protein expression was analyzed by standard biotin-avidin immunohistochemical analysis using a polyclonal mouse antibody to GP73. Protein expression was evaluated on a wide range of prostate tissue using high-density tissue microarrays. High levels of staining were observed in prostate cancer tissue. Some normal epithelial cells did not stain for GP73 in a sub region of prostate cancer tissue.

Figure 32 shows immunoblot analysis of normal and prostate cancer epithelial cells. The epithelial cells were isolated from normal prostate tissue and cancer tissue to specifically isolate the protein from epithelial cell for GP73 immunoblot analysis. For this purpose, laser capture microdissected samples were used. Actin western serves as control.

Example 12

Lethal Markers and Targets

This example describes the identification of lethal markers. The markers serve as potential therapeutic targets. Markers were identified by correlating the number of samples with clinical parameters and gene expression. Specifically, the present study identified markers that have an expression profile similar to EZH2, which serves as a prototypic lethal biomarker of prostate cancer. These genes were identified by a scoring

system that takes into account whether localized prostate cancer has recurred or not recurred. In addition, genes that have highly correlated expression with EZH2 were identified that may serve as markers to supplement EZH2.

5

		Total	16	13	16	6	20			
mean	dev	High	bph_count	pca_count	pcau_count	pcar_count	met_count	score	UNIQID	NAME
-0.024	0.3725	0.7206	0	4	5	6	16	18	5814	NULL ESTs Hs.30237
-0.306	0.1707	0.0351	0	0	3	3	14	17	2506	HN1
-0.348	0.2394	0.1312	0	2	1	4	14	16	5112	CSF2
0.0623	0.1578	0.3779	0	1	2	3	13	15	6053	ASNS
-0.246	0.1689	0.0921	0	2	0	2	15	15	1520	NULL ESTs Hs.16304
-0.212	0.1386	0.0648	0	2	0	2	15	15	8273	PRC1
-0.352	0.1458	-0.06	0	3	7	3	14	14	34	GPAA1
-0.292	0.2538	0.2153	0	0	1	3	10	13	5239	KIAA1691
-0.141	0.1572	0.1729	0	2	5	3	12	13	8562	NULL Human clone 23614
-0.21	0.1083	0.0067	0	4	4	2	15	13	3351	FLJ11715 hypothetical protein
-0.22	0.1846	0.1495	0	5	4	5	13	13	2715	NULL ESTs
-0.638	0.2696	-0.099	1	5	4	3	15	13	9556	FLJ12443 hypothetical protein
-0.142	0.1396	0.1371	0	0	2	2	10	12	1158	TGFBI
-0.124	0.1606	0.1967	0	1	1	3	10	12	5292	NULL ESTs
-0.444	0.2474	0.0504	0	1	2	2	11	12	3689	NUF2R hypothetical protein
-0.205	0.2362	0.2674	0	2	1	2	12	12	1219	ABCC5
-0.09	0.2214	0.3526	0	4	2	4	12	12	1360	MEN1
-0.241	0.1541	0.0673	0	5	3	2	15	12	8476	SARM and HEAT/Armadillo motif
-0.874	0.3367	-0.201	0	1	4	2	10	11	3747	H2BFB
-0.196	0.254	0.3122	0	2	1	3	10	11	4941	VAV2
-0.166	0.1486	0.1307	0	2	4	2	11	11	8636	NULL ESTs Hs.23268
0.0255	0.1542	0.3338	0	3	3	3	11	11	280	TOP2A
-0.226	0.2536	0.2812	0	4	3	4	11	11	2156	EZH2
-0.031	0.1826	0.3346	0	4	4	2	13	11	1979	NULL ESTs Hs.268921
-0.48	0.2967	0.1131	0	2	0	2	10	10	906	MGC5627 hypothetical protein
-0.243	0.1421	0.0411	0	2	8	2	10	10	3728	NULL ESTs
-0.133	0.1806	0.2279	0	2	2	2	10	10	8759	RAB24
-0.192	0.1782	0.1645	0	3	2	2	11	10	2029	FLJ12876 hypothetical protein
-0.617	0	-0.617	0	3	2	2	10	9	3928	DGKD
0.1079	0.1132	0.3343	0	3	2	2	10	9	5372	ODF2
-0.288	0.1221	-0.043	0	4	3	3	10	9	7193	KIAA0602
-0.167	0.2278	0.2883	0	4	2	2	11	9	8535	EHM2

-0.95	0.3504	-0.249	0	4	2	2	11	9	9824	SLC19A1
-0.314	0.187	0.06	1	4	2	2	11	9	9447	LIG1
0.1366	0.1883	0.5132	1	4	3	2	10	8	327	NULL ESTs
-0.586	0.2952	0.0044	0	5	2	2	11	8	1269	DGKZ

mean: mean expression in BPH
Dev: standard deviation in BPH
High: 2 SD's above the mean (threshold)
Bph: # of BPH samples > thresh
PCA: # of PCA samples > thresh (>1yr no recur)
Pcau: # of PCA samples > thresh (<1yr followup)
Pcar: # of PCA samples > thresh (recur)
Met: # of metastatic samples > thresh
Score: = met + pcar - pca
Total: # of samples in category

Exemplary lethal markers identified using the above methods include ABCC5 (MDR5). This multi-drug resistance gene actively pumps cyclic nucleotides and other small molecules out of cells. An unrelated study found that this enzyme is potently Inhibited by phosphodiesterase inhibitors, including sildenafil (viagra). The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not required to practice the present invention. Nonetheless, it is contemplated that sildenafil may be useful in the treatment of aggressive PCA.

Another lethal marker identified is asparagine synthetase (ASNS). Current therapeutics for the inhibition of ASNS include asparaginase, an enzyme that destroys asparagine in the body. It has been shown that cancers expressing the synthetase are resistant. Analogs are being developed to inhibit the synthetase.

Top2A (topoisomerase 2) and the Vav2 Oncogene were also identified using the methods of the present invention. Vav2 is required for cell spreading, but is dependent on src. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not required to practice the present invention. Nonetheless, it is contemplated src inhibitors can stop vav2 mediated cell spreading

This example describes the identification of cancer markers overexpressed in prostate cancers. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention.

Nonetheless, it is contemplated that therapeutic compounds that inhibit these lethal markers are useful in the treatment of prostate cancer.

Example 13

Characterization of Annexin Expression in Prostate Cancer

This Example describes the expression of Annexins in prostate cancer.

A. Materials and Methods

Prostate Sample Collection

Prostate tissues were taken from the radical prostatectomy series and the rapid autopsy program available through the University of Michigan Prostate Cancer Specialized Program of Research Excellence (S.P.O.R.E.) Tissue Core. This program is approved by Institutional Review Board at the University of Michigan.

Hormone naïve, clinically localized PCA samples used for this study were taken from a cohort of men who underwent radical retropubic prostatectomy as a monotherapy (*i.e.*, no hormonal or radiation therapy) for clinically localized PCA between the years 1994 and 1998. Processing of the prostatic tissues started within 20 minutes after surgical resection. The prostates were partially sampled and approximately 50% of the tissue was used for research. This protocol has been evaluated in a formal study to assure that partial sampling does not impair accurate staging and evaluation of the surgical margins (Hollenbeck *et al.*, J. Urol. 164:1583 [2000]). The snap frozen samples used for cDNA expression array analysis were all evaluated by one of the study pathologists. All samples were grossly trimmed to ensure greater than 95% of the sample represented the desired lesion.

Hormone refractory PCA samples were collected from the rapid autopsy program (Rubin *et al.*, [2000], *supra*). Snap frozen samples were used for cDNA expression array analysis. Mirrored samples from the same lesion were placed in 10% buffered formalin. The fixed samples are embedded in paraffin. As with the prostatectomy samples, the study pathologist reviewed the glass slides, circled areas of viable prostate cancer, avoiding areas of necrosis, and used these slides as a template for tissue microarray construction. In this study, twenty (20) hormone refractory metastatic PCAs were

extracted from 15 rapid autopsy cases performed from 1997 to 2000. The patients' ages ranged from 53 to 84 and time from diagnosis to death ranged from 21 to 193 months. All 15 patients died with widely metastatic PCA after extensive treatment, which included antiandrogens and chemotherapy.

Prostatectomy samples were evaluated for the presence or absence of surgical margin involvement by tumor (surgical margin status), the presence of extraprostatic extension, and seminal vesicle invasion. Tumors were staged using the TNM system, which includes extraprostatic extension and seminal vesicle invasion but does not take into account surgical margin status (Bostwick *et al.*, Semin. Urol. Oncol. 17:222 [1999]). Tumors were graded using the Gleason grading system (Gleason, [1966], *supra*).

Immunohistochemistry

After paraffin removal and hydration, the tissue microarray slides were immersed in 10 mM citrate buffer placed in a pressure cooker chamber and microwaved for 10 minutes for optimal antigen retrieval. Immunostaining was performed using a Dako autostainer (DAKO, Carpinteria, CA). The primary antibody was incubated for 45 minutes at room temperature and a secondary biotin-labeled antibody for 30 minutes. Streptavidin-LSA amplification method (DAKO K0679) was carried out for 30 minutes followed by peroxidase/diaminobenzidine substrate/Chromagen. The slides were counterstained with hematoxylin. Polyclonal antibodies directed against the N-terminus of annexin 1(dilution 1:50), annexin 2 (dilution 1:100), annexin 4 (dilution 1:100), annexin 7 (dilution 1:500), and annexin 11 (dilution 1:100) were obtained from a signal source (Santa Cruz Biotechnology, Santa Cruz, CA). Protein expression as determined by two pathologists immunohistochemistry was scored as negative (score=1), weak (score 2), moderate (3) or strong (4), using the system described above.

Tissue Microarray Construction, Digital Image Capture, and Analysis

Tissue microarrays were constructed as previously described to evaluate protein expression in a wide range of samples ranging from benign prostate tissue taken from the prostatectomy samples to hormone refractory PCA. Three tissue microarrays were used for this study consisting of benign prostate, localized PCAs, and hormone refractory

PCA. The tissue microarrays were assembled using the manual tissue arrayer (Beecher Instruments, Silver Spring, MD) as previously described (Kononen *et al.*, [1998], *supra*; Perrone *et al.*, [2000], *supra*). Tissue cores from the circled areas of interest were targeted for transfer to the recipient array blocks. The 0.6mm diameter tissue microarray cores were each spaced at 0.8mm from core-center to core-center. Tissue microarray images were acquired using the BLISS Imaging System (Bacus Lab, Lombard, IL).

Statistical Analyses

To investigate the statistical significance associated with the differential expression of annexins across 4 independent gene expression studies, standard methods (Hedges *et al.*, Statistical Methods for Meta-analysis meta-analysis. Orlando, Academic Press 1985, pp xxii, 369) were used to combine the results. For each of the studies, a t-statistic was computed (with the two groups being benign tissue compared against localized prostate cancer) and the associated p-values were transformed using a negative logarithmic transformation. These numbers were then doubled and added together to arrive at a summary measure of differential gene expression across the three studies. To assess the statistical significance associated with this summary measure, a permutation-based approach was adopted (Hedges *et al.*, *supra*). Namely, the tissue types were permutated within studies, and the summary measure was computed for the permutated data. A p-value was computed using the permutation distribution of the summary measure. The issue then arises of whether or not the t-statistics from the three studies are comparable.

Annexin protein expression was statistically evaluated using the mean score results from each tissue microarray sample for each prostate tissue type (*i.e.*, benign, localized PCA, and hormone refractory PCA). To determine differences between all pairs (*e.g.*, localized prostate cancer versus benign), an ANOVA with a post-hoc analysis was performed using the Scheffé method (Scheffae *et al.*, *supra*). The mean expression scores for all examined cases were presented in a graphical format by using error-bars with 95% confidence intervals.

B. Results

Expression array analysis revealed a significant dysregulation of annexin family members with PCA progression. The cDNA expression of annexins 1, 2, 4, 7 and 11 were significantly decreased in the hormone refractory PCA samples as compared to localized hormone sensitive PCA samples with 2.2, 1.5, 1.3, 1.4 and 1.8 fold decrease, respectively (all p-values < 0.01) (Table 3 and Figure 33). Annexins 1 and 4 showed significant decreases of mRNA expression in localized PCA samples as compared to the benign samples. There were no significant differences between localized hormone naive PCA and the benign samples for annexin 2, 7, and 11. No cDNA dysregulation between the tested prostate samples and annexins 8 and 13 was observed. Annexin 6 demonstrated a slight decrease in cDNA expression between localized PCA and benign samples, which was not statistically significant (Table 3).

In order to cross validate the cDNA expression results for these annexin family members, a meta-analysis of gene expression was performed. Annexin family members cDNA expression results were evaluated using a series of data sets (Welsh *et al.*, Cancer Res. 61:5974 [2001]; Luo *et al.*, Cancer Res. 61:4683 [2001]; Magee *et al.*, Cancer Res. 61:5692 [2001]). The analysis evaluated annexins for each of the individual studies as well as performing a summary statistic, taking into account the significance of the gene expression across the 4 studies. The meta-analysis compared differences between clinically localized PCA and benign prostate tissue as not all of the studies had hormone refractory metastatic PCA. The meta-analysis (Table 4 and Figure 34) demonstrated that annexins 1, 2, 4, and 6 were significantly down regulated across independent studies. Annexin 6 was down regulated to a significant level in 4 of 4 studies. Annexin 1 demonstrated down regulation in 3 of 4 studies. Annexins 2 and 4 were down regulated in 2 studies and overall considered to be significantly under expressed by the meta-analysis. Annexin 7 was not found to be significantly under expressed in any of the 4 studies at the transcript level.

Immunohistochemistry was performed to confirm these results at the protein level (Table 5). By immunohistochemistry, a significant decrease in protein expression for annexins 1, 2, 4, 7 and 11 in hormone refractory PCA samples as compared to localized PCA samples was identified with 2.5 (3.8 vs. 1.5 median expression), 2.4 (4 vs. 1.7

median expression), 3.6 (4 vs. 1.1 median expression) and 3.3 (4 vs. 1.2 median expression) fold decreases, respectively (Kruskal Wallis test, all p-values $p < 0.05$). No statistically significant differences were seen between benign and localized PCA samples in any of the annexins tested.

Table 3: Gene Expression of Select Annexins.

Annexin	Benign		BPH[1]		Loc- PCA[2]		Met-PCA[3]		**Ratio**	p
	Count	Median	Count	Median	Count	Median	Count	Median	**PCA/Met**	Value*
1	5	1.56	16	1.35	16	0.69	20	0.31	**2.23**	<0.001
2	5	0.79	16	0.69	16	0.74	20	0.49	**1.51**	0.009
4	5	0.91	16	0.97	16	0.9	20	0.69	**1.30**	0.001
6	5	1.2	16	1.29	16	1.05	20	1.15	**0.91**	0.377
7	5	0.8	16	0.88	16	0.88	20	0.62	**1.42**	<0.001
8	5	1.14	16	1.06	16	0.99	20	1.19	**0.83**	0.156
11	5	0.99	16	0.76	16	0.94	20	0.52	**1.81**	<0.001
13	5	1.08	16	1.35	16	1.03	20	0.94	**1.10**	0.393

* Kruskal Wallis Test. 1, BPH, benign prostatic hyperplasia. 2, Loc-PCA, localized prostate cancer. 3, Met-PCA, metastatic hormone refractory prostatic cancer. Ratio PCA/Met, ratio of expression of localized PCA over hormone refractory PCA.

Table 4: Meta-Analysis of cDNA Prostate Gene Expression Studies for Annexin Family Members

Annexin	Present study	Welsh *et al.*	Luo *et al.*	Magee *et al.*	Summary p-Value
6	**0.024**	**0.0001**	**0.0001**	**0.026**	**0.0001**
1	**0.0001**	**0.031**	**0.0007**	0.23	**0.0001**
2	NA	**0.0001**	NA	**0.002**	**0.0001**
11	NA	**0.010**	NA	0.6	0.17
7	0.25	0.48	0.38	0.088	0.20
4	0.33	**0.023**	**0.0093**	0.58	**0.011**

13	0.177	NA	1.00	NA	0.48
8	0.79	NA	0.104	NA	0.29

Table 5: Tissue Microarray Protein Expression for Annexins by Tissue Type

Annexin	Benign		Loc-PCA[2]		Met-PCA[3]		PCA/MET	p-value*
	Count	Median	Count	Median	Count	Median		
1	37	2.59	360	2.45	162	1.46	1.68	<0.001
2	57	3.95	82	3.62	214	1.47	2.46	<0.001
4	23	3.65	357	3.96	141	1.57	2.52	<0.001
7	26	3.77	350	3.97	126	1.32	3.01	<0.001
11	23	4.00	360	3.99	163	1.30	3.01	<0.001

* Kruskal Wallis Test. 1, BPH, benign prostatic hyperplasia. 2, Loc-PCA, localized prostate cancer. 3, Met-PCA, metastatic hormone refractory prostatic cancer.

Example 14

Association of CtBP with Prostate Cancer

This example describes the expression of C-terminal binding proteins 1 and 2 (CtBP1 and CtBP2) in prostate cancer. Microarray analysis, Western Blots, immunohistochemistry, and statistical analysis were performed as described in the above examples.

The CtBP transcript was found to be up-regulated in metastatic prostate cancer (Figure 38). Tissue extracts were used to validate this finding at the protein level using an antibody that recognizes CtBP1 and CtBP2 (Sewalt *et al.*, Mol. Cell. Biol. 19:777 [1999]. The results are shown in Figure 35. Figure 35 shows the Expression of CtBP proteins in PCA specimens. Extracts from selected prostate specimens were assessed for expression of CtBP and PcG proteins by immunoblot analysis. Protein level was equalized in each extract before loading and blots were stained with Ponceau S to confirm equal loading. β-tubulin was used as a control protein.

Both CtBPs were over-expressed in metastatic prostate cancer relative to localized prostate cancer and benign tissue. EZH2 protein was also elevated in metastatic prostate cancer relative to localized prostate cancer or benign prostate (Figure 35). EED, a PcG protein that forms a complex with EZH2, along with an un-related protein, β-tubulin, did not exhibit similar protein dysregulation. Thus, both transcriptional repressors (CtBP and EZH2) are mis-expressed in metastatic prostate cancer.

To determine *in situ* expression of CtBP, immunohistochemistry of prostate tissue sections were performed using prostate tissue microarrays. Benign prostatic epithelia exhibited exclusively nuclear staining consistent with CtBP's role as a transcriptional repressor. Both clinically localized and metastatic prostate cancer exhibited nuclear staining as well. Most of the metastatic prostate cancer cases and a fraction of the localized prostate cancer cases exhibited distinct cytoplasmic staining of CtBP.

Figure 36 shows tissue microarray analysis of CtBP in prostate cancer that suggests mis-localization during prostate cancer progression. The mean CtBP protein expression for the indicated prostate tissues and sub-cellular compartment is summarized using error bars with 95% confidence intervals. Figure 37 shows the sub-cellular fractionation of LNCaP cells. The results show an increased level of CtBP1 in the cytoplasm relative to the nucleus. CtBP2 is weakly expressed in the cell lines and is not easily apparent. β-tubulin, which is not expressed in the nucleus, is provided as a control. Figure 38 shows a Kaplan-Meier Analysis of prostate cancer tissue microarray data. The results demonstrate that the presence of cytoplasmic CtBP may be associated with a poorer clinical outcome. The median follow up time for all patients was 1 year (range 2 month to 6.5 years). Over this follow up time, 38% of the patients developed a recurrence or PSA elevation greater than 0.2 ng/ml. Prostate tumors from 97 patients demonstrated near uniform nuclear protein expression for CTBP. Cytoplasmic expression was variable with 85 of 97 cases (88%) demonstrating weak cytoplasmic staining and 12 (12%) with moderate to strong CTBP expression. There was a significant association with increased CTBP cytoplasmic staining intensity and PSA recurrence or presence of recurrent disease following prostatectomy with a relative risk of 1.7 (Cox regression analysis p=0.034). The data presented demonstrates a Kaplan-Meier Analysis of outcome stratified by negative/weak cytoplasmic CTBP staining and moderate/strong

staining. CTBP cytoplasmic expression predicted recurrence even when Gleason score was taken into account in a multivariable model, suggesting that CTBP is a prognostic predictor of poor outcome [Gleason relative risk 1.4 (p=0.005) and cCTBP rr 1.6 (p=0.042)].

CtBP has been shown to bind nitric oxide synthase (NOS), which is thought to shift the localization of CtBP from the nuclear compartment to the cytoplasmic compartment (Riefler *et al.*, J. Biol. Chem. 276:48262 [2001]). Weigert and colleagues have proposed a cytoplasmic role for CtBP in the induction of Golgi membrane fission (Weigart *et al.*, Nature 402:429 [1999]). To further support the preliminary immunohistochemical findings, LNCaP (metastatic) prostate cancer cells were fractionated and it was found that CtBP levels were higher in the cytosol relative to the nucleus (Figure 38).

Example 15

Methods of Characterizing Cancer Markers

This example describes exemplary methods for the characterization of new cancer markers of the present invention. These methods, in combination with the methods described in the above examples, are used to characterized new cancer markers and identify new diagnostic and therapeutic targets.

A. Determination of quantitative mRNA transcript levels of cancer markers in prostate cancer specimens

In some embodiments, markers revealed to be over or under expressed in cancer microarrays (*See e.g.*, Example 1 for a description of microarrays) are quantitated using real-time PCR (Wurmbach *et al.*, J. Biol. Chem. 276:47195 [2001]).

In preferred embodiments, cDNA from over 100 prostate samples for archived cDNA samples and associated clinical data are available (See Example 1). The level of expression in the microarray is compared to those obtained by real-time PCR. To identify genes with dysregulation of expression, real-time PCR analysis of cDNA generated from laser-capture microdissected prostate cancer epithelia and benign epithelia is performed.

B. Detection of Mis-localized Transcripts

In some embodiments, in order to determine if a cancer marker normally present in the nucleus of a cell (*e.g.*, a transcriptional repressor) is mis-localized to the cytoplasm (or other mis-locations) in cancer, the expression of the marker is examined in tissue extracts from preferably at least 20 benign prostate samples, 20 prostate cancer specimens, and 20 metastatic prostate specimens. Expression of the marker in benign prostate cell lines (RWPE), primary prostatic epithelial cells (Clonetics, Inc.) and a panel of prostate cancer cells including LNCaP, DU145, PC3, DUCaP, and VCaP cells is also examined. Once overall expression of prostate cell lines and tissues is established, the cellular localization of the marker is determined by 2 methods. In the first method, the cell and tissue extracts are fractionated into a nuclear fraction and a cytosolic fraction (NE-PER, Pierce-Endogen; Orth *et al.*, J. Biol. Chem. 271:16443 [1996]). Quantitated protein is then analyzed by immunoblotting. Relative levels of cytosolic and nuclear cancer marker are determined by densitometry. To verify clean fractionation, antibodies to β-tubulin and PCNA (or lamin A) are used to assess cytosolic and nuclear fractions, respectively.

In the second method, cells are immunostained with antibodies to the cancer marker followed by detection using anti-rabbit FITC secondary antibody. Confocal microscopy (U of M Anatomy and Cell Biology Core Facility) is used to examine *in situ* localization of the cancer markers.

In some embodiments, mis-localization is further investigated by sequencing the gene in cells containing the mis-located transcript (*e.g.*, metastatic cases) for mutations.

C. Correlation of cancer markers with clinical outcome

In some preferred embodiments, the association of expression or mis-localization of a cancer marker with clinical outcome is investigated. The ratio of total cancer marker to β-tubulin by immunoblot analysis of prostate cancer tissue extracts is first determined and associated with clinical outcome parameters. For markers suspected of being mis-localized in cancer (*e.g.*, CtBP), the ratio of cytoplasmic marker to nuclear marker is next determined by immunoblot analysis of prostate cancer tissue extracts and associated with clinical outcome parameters. For example, it is contemplated that a high

cytoplasmic/nuclear cancer marker ratio may portend a poor clinical outcome. In some embodiments (*e.g.*, where a cancer marker is suspected of being mis-localized), immunohistochemistry of prostate cancer tissue microarrays is used to determine whether the presence of cytoplasmic marker correlates with poor clinical outcome. Tissue microarrays are prepared and performed as described in the above examples.

Briefly, high-density tissue microarrays (TMA) are constructed as previously described (Perrone et al, *supra*; Kononen *et al.*, *supra*). Immunostaining intensity is scored by a genitourinary pathologist as absent, weak, moderate, or strong (or alternatively analyzed separately as for cytoplasmic and nuclear staining). Scoring is performed using a telepathology system in a blinded fashion without knowledge of overall Gleason score (*e.g.*, tumor grade), tumor size, or clinical outcome (Perrone *et al.*, *supra*). Tumor samples are derived from patients with clinically localized, advanced hormone refractory prostate cancer and naïve metastatic PCA. Cases of clinically localized prostate cancer are identified from the University of Michigan Prostate S.P.O.R.E. Tumor Bank. All patients were operated on between 1993 and 1998 for clinically localized prostate cancer as determined by preoperative PSA, digital-rectal examination, and prostate needle biopsy. All tissues used are collected with institutional review board approval. The advanced prostate tumors are collected from a series of 23 rapid autopsies performed at the University of Michigan on men who died of hormone refractory prostate cancer. The clinical and pathologic findings of these cases have been reported (Rubin *et al.*, [2000], *supra*).

Statistical analysis of the array data is used to correlate the cancer marker protein measurements on the TMA with clinical outcomes, such as time to PSA recurrence and survival time. This analysis involves survival analysis methods for correlating the measurements with these censored response times. Kaplan-Meier curves are plotted for descriptive purposes. Univariate analyses is performed using the Cox model associating the biomarker with the survival time. In addition, multivariate Cox regression analysis is performed to test whether the biomarker adds any prognostic information over and above that available from known prognostic markers (*i.e.*, Gleason score, tumor stage, margin status, PSA level before surgery).

D. RNA Interference

In some embodiments, RNA interference of cancer markers is used to investigate the role of the cancer marker in cell culture and well as for application as a therapeutic cancer treatment (See *e.g.*, Example 8 for an example of RNA interference). 21-nucleotide RNAs (siACE-RNAi) are synthesized through a commercial vendor (Dharmacon Research, Inc.). RNA interference has been used in mammalian cells (Elbashir *et al.*, Nature 411:494 [2001]). Several siRNA duplexes and controls are designed for each marker. The design of the siRNA duplexes uses criteria provided by Elbashir *et al.* (Elbashir *et al.*, *supra*) and Dharmacon Research which include: starting approximately 75 bases downstream of the start codon, locating an adenine-adenine dimer, maintaining G/C content around 50%, and performing a BLAST-search against EST databases to ensure that only one gene is targeted. Multiple (*e.g.*, two) siRNA duplexes are designed for each molecule of interest since whether the siRNA duplex is functional is a relatively empirical process. In addition, it is contemplated that using two siRNA duplexes may provide a combined "knock-down" effect. As a control, a "scrambled" siRNA, in which the order of nucleotides is randomized, is designed for each molecule of interest. Oligonucleotides are purchased deprotected and desalted. Upon arrival, the oligonucleotides are annealed to form a duplex using the manufacturer's provided protocol.

To test the efficacy of each siRNA duplex, prostate cell lines (RWPE, DU145, LnCAP, and PC3) are transfected with the OLIGOFECTAMINE reagent as described (Elbashir *et al.*, *supra*). The cells are assayed for gene silencing 48 hrs post-transfection by immunoblotting with respective antibodies. A number of controls are included: buffer controls, sense siRNA oligo alone, anti-sense siRNA oligo alone, scrambled siRNA duplex, and siRNA duplexes directed against unrelated proteins. If significant silencing is not appreciated after single transfection, sequential transfection is performed and inhibition is monitored at later time points (*i.e.*, 8 days later) as suggested by others (Breiling *et al.*, Nature. 412: 51 [2001]). This may be necessary with proteins that have a long half-life.

In addition to the transient expression of siRNAs, a method for stable expression of siRNAs in mammalian cells is used (Brummelkamp *et al.*, Science 296:550 [2002]).

Prostate cancer cell lines are generated that express siRNA targeting cancer markers using the pSUPER system. Scrambled siRNA is used as a control. The cell lines facilitate downstream characterization of cancer markers that may be cumbersome using duplexes transiently. If inhibition of a specific cancer marker is found to be toxic to cells, the pSUPER cassette containing siRNA to the marker is cloned into an inducible vector system (*e.g.*, Tet on/off).

E. Generation of Mutants.

To study the function of cancer markers of the present invention, mutants of cancer markers are generated in eukaryotic expression vectors. myc-epitope tagged versions of cancer marker mutants are generated in both pcDNA3 and pcDNA3-ER (a modified estrogen receptor ligand binding domain). In the case of the ER constructs, the vectors produce an in-frame fusion protein with modified ER, thus generating a post-transcriptionally inducible vector (Littlewood *et al.*, Nucleic Acids Res. 23: 686 [1995]). The ER-ligand domain is mutated and fails to bind endogenous estrogen, yet can be activated by 4-hydroxytamoxifen (Littlewood *et al.*, *supra*). The ER-fusion proteins are inactivated in the absence of ligand presumably due to binding of proteins such as hsp90. In the presence of exogenously added 4-hydroxytamoxifen, ER-fusions become liberated. By using an inducible vector system, cell lines expressing a "toxic" or growth inhibitory version of a cancer marker can still be isolated.

Various N-terminal and C-terminal deletion mutants are generated that encompass function domains of the cancer marker (*e.g.*, the PXDLS, dehydrogenase, and PDZ binding domains of CtBP; Chinnadurai, Mol Cell. 9: 213 [2002]). It is contemplated that some of the mutant versions of the cancer markers of the present invention act as dominant negative inhibitors of endogenous cancer marker function. Expression of epitope-tagged cancer markers and mutants is assessed by transient transfection of human embryonic kidney cells (using FUGENE) and subsequent Western blotting.

F. Establishing Stable Cell Lines Expressing Cancer Markers And Mutants

In some embodiments, cell lines stably expressing cancer markers of the present invention are generated for use in downstream analysis. FUGENE is used to transiently transfect prostate cell lines (RWPE, DU145, LnCAP, and PC3) with cancer markers and

fusions or mutants using the above mentioned vectors and appropriate G418 selection. Prostate cell lines with varied expression levels of endogenous cancer marker protein are used. Both individual clones and pooled populations are derived and expression of cancer markers and mutants assessed by immunoblotting for the epitope tag. By also using an inducible system, clones expressing toxic versions of cancer markers or mutants can be isolated.

G. Cell proliferation and apoptosis studies

In some embodiments, the role of cancer marker expression in prostate cell proliferation is investigated using a multi-faceted approach that includes 1. RNA interference, 2. transient transfection of cancer markers and potential dominant negative mutants, and 3. comparing stable transfectants of cancer markers and mutants. The following predictions are tested using these methods: 1. whether inhibition of cancer markers will block cell growth and 2. whether overexpression of cancer markers will enhance cell proliferation.

Cell proliferation is assessed by cell counting (Coulter counter) over a time course in culture by using the WST-1 reagent (Roche, Inc.), which is a non-radioactive alternative to [^{3}H]-thymidine incorporation and analogous to the MTT assay. The rate of incorporation of the DNA labeling dye bromodeoxyuridine (BrdU) will also be measured as described previously (Jacobs *et al.*, Nature. 397:164 [1999]). Potential cell cycle arrest induced by siRNA or dominant negative inhibitors of is determined by conventional flow cytometric methods. By using stable cell lines that "activate" cancer markers and mutants in a 4-hydroxytamoxifen-dependent fashion, cell proliferation and cell cycle alterations are monitored in a highly controlled *in vitro* system. To confirm that overexpression or inhibition of cancer markers does not activate the apoptosis pathway, several assays are used including propidium iodide staining of nuclei, TUNEL assay and caspase activation.

If a cancer marker is found to be a regulator of cell proliferation in prostate cells, studies are designed to address how components of cell cycle machinery are modulated by the cancer marker. Thus, in order to study cancer marker mediated effects on the cell cycle machinery of prostate cells, cancer marker functions are modulated with the above

mentioned tools (*i.e.*, siRNA, dominant negative inhibition, etc.) and the expression levels (transcript and protein) of cyclins (cyclin D1,E,A), cyclins-dependent kinases (cdk2, cdk4, cdk6) and cyclin-dependent kinase inhibitors (p21CIP1, p27KIP1, p45SKP2, p16INK4) are monitored.

H. Cell Adhesion and Invasion Assays

If a cancer marker is suspected of altering cell adhesion (*e.g.*, the transcriptional repression of an epithelial gene program such as E-cadherin), the methods described above are used to investigate whether over-expression of the cancer marker causes increased or decreased cell adhesion. Adhesion to extracellular matrix components, human bone marrow endothelium (HBME) as well as to human umbilical vein endothelial cells (HUVEC) is tested. Cancer markers are further tested for their ability to modulate invasion of PCA.

Known methods are used in these studies (Cooper *et al.*, Clin. Cancer Res. 6:4839 [2000]). Briefly, snap-apart 96-well tissue culture plates are coated with crude bone and kidney matrices. Plates are incubated overnight at room temperature under sterile conditions and stored at 4°C until needed. Assay plates are also coated with extracellular matrix components (*e.g.*, human collagen I, human fibronectin, mouse laminin I) and human transferrin at various concentrations according to the manufacturer's instruction (Collaborative Biomedical Products, Bedford, MA). Endothelial cells (HBME or HUVEC) are seeded onto bone matrices or plastic substrata at a concentration of 900 cells/μl and grown to confluence. Tumor cells are removed from the flask by a 15-20 minute treatment with 0.5mM EDTA in Hank's balanced salt solution. Once the EDTA solution is removed, the cells are resuspended in adhesion medium (*e.g.*, minimum essential medium (MEM) with 1% bovine serum albumin (BSA) supplemented with 10 uCi ^{51} Cr sodium salt (NEN, Boston, MA)) for 1 hour at 37°C. Cells are then washed three times in isotope free media and 1 x 10^5 radio-labeled tumor cells are resuspended in adhesion media and layered upon a confluent layer of endothelial cells for 30 min at 37°C. In addition, radiolabeled tumor cells are applied to crude bone matrices. Again, plates are washed three times in phosphate buffered saline and adhesion is determined by

counting individual wells on a gamma counter. Cell adhesion is reported relative to the adhesion of controls (PC-3 cells on plastic), which are set to 100.

Cell invasion assays are performed using a classic Boyden chamber assay. Both strategies to inhibit and overexpress cancer markers are evaluated. Previous reports have correlated increased cell migration in a Boyden Chamber system with increased invasive properties *in vivo* (Klemke *et al.*, J Cell Biol. 140:61 [1998]. Commercially available 24-well invasion chambers are used (*e.g.*, BD biosciences, Chemicon International).

I. Transcriptional Suppression in Prostate Cancer Cells

In some embodiments, the effect of cancer markers on gene silencing in prostate cells is assessed. Gene silencing is assayed in several ways. First, gene expression alterations induced by transient transfection of cancer markers and mutants in prostate cell lines (RWPE, DU145, LnCAP, and PC3) is assayed using FUGENE. Twelve to 48 hours after transfection, cells are harvested and a portion is processed to confirm expression of the transfectants by immunoblotting. Using vector-transfected cells as a reference sample, total RNA from transfected cells is then assessed on 20K cDNA microarrays.

In addition to transient transfections, stable cell lines overexpressing cancer markers and cancer marker mutants are generated. Patterns of gene expression from cancer marker and cancer marker mutant expressing cell lines are compared to vector-matched controls in order to identify a gene or group genes that is repressed by a given cancer marker. The present invention is not limited to a particular mechanism. Indeed, and understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that genes identified as repressed by a given cancer marker will be increased (de-repressed) upon knock-down of the cancer marker (*e.g.*, by siRNA inhibition).

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in

connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

CLAIMS

We claim:

1. A method for characterizing prostate tissue in a subject, comprising:

a) providing a prostate tissue sample from a subject; and

b) detecting the presence or absence of expression of hepsin in said sample, thereby characterizing said prostate tissue sample.

2. The method of Claim 1, wherein said detecting the presence of expression of hepsin comprises detecting the presence of hepsin mRNA.

3. The method of Claim 2, wherein said detecting the presence of expression of hepsin mRNA comprises exposing said hepsin mRNA to a nucleic acid probe complementary to said hepsin mRNA.

4. The method of Claim 1, wherein said detecting the presence of expression of hepsin comprises detecting the presence of a hepsin polypeptide.

5. The method of Claim 4, wherein said detecting the presence of a hepsin polypeptide comprises exposing said hepsin polypeptide to an antibody specific to said hepsin polypeptide and detecting the binding of said antibody to said hepsin polypeptide.

6. The method of Claim 1, wherein said subject comprises a human subject.

7. The method of Claim 1, wherein said sample comprises tumor tissue.

8. The method of Claim 7, wherein said tumor tissue sample is a post-surgical tumor tissue sample and said method further comprises the step of c) identifying a risk of prostate specific antigen failure based on said detecting the presence or absence of expression of hepsin.

9. The method of Claim 1, wherein said characterizing said prostate tissue comprises identifying a stage of prostate cancer in said prostate tissue.

10. The method of Claim 9, wherein said stage is selected from the group consisting of high-grade prostatic intraepithelial neoplasia, benign prostatic hyperplasia, prostate carcinoma, and metastatic prostate carcinoma.

11. The method of Claim 1, further comprising the step of c) providing a prognosis to said subject.

12. The method of Claim 11, wherein said prognosis comprises a risk of developing prostate specific antigen failure.

13. The method of Claim 12, wherein said prognosis comprises a risk of developing prostate cancer.

14. A method for characterizing prostate tissue in a subject, comprising:

a) providing a prostate tissue sample from a subject; and

b) detecting the presence or absence of expression of pim-1 in said sample, thereby characterizing said prostate tissue sample.

15. The method of Claim 14, wherein said detecting the presence of expression of pim-1 comprises detecting the presence of pim-1 mRNA.

16. The method of Claim 15, wherein said detecting the presence of expression of pim-1 mRNA comprises exposing said pim-1 mRNA to a nucleic acid probe complementary to said pim-1 mRNA.

17. The method of Claim 14, wherein said detecting the presence of expression of pim-1 comprises detecting the presence of a pim-1 polypeptide.

18. The method of Claim 17, wherein said detecting the presence of a pim-1 polypeptide comprises exposing said pim-1 polypeptide to an antibody specific to said pim-1 polypeptide and detecting the binding of said antibody to said pim-1 polypeptide.

19. The method of Claim 14, wherein said subject is a human subject.

20. The method of Claim 14, wherein said sample comprises tumor tissue.

21. The method of Claim 20, wherein said tumor tissue is post-surgical tumor tissue and said method further comprises the step of c) identifying a risk of prostate specific antigen failure based on said detecting the presence or absence of expression of hepsin.

22. The method of Claim 14, wherein said characterizing said prostate tissue comprises detecting a stage of prostate cancer in said prostate tissue.

23. The method of Claim 22, wherein said stage is selected from the group consisting of high-grade prostatic intraepithelial neoplasia, benign prostatic hyperplasia, prostate carcinoma, and metastatic prostate carcinoma.

24. The method of Claim 14, further comprising the step of c) providing a prognosis to said subject.

25. The method of Claim 24, wherein said prognosis comprises a risk of developing prostate specific antigen failure.

26. The method of Claim 24, wherein said prognosis comprises a risk of developing prostate cancer.

27. A method for characterizing prostate tissue in a subject, comprising:

a) providing a prostate tissue sample from a subject; and

b) detecting the presence or absence of expression of EZH2 in said sample, thereby characterizing said prostate tissue sample.

28. The method of Claim 27, wherein said detecting the presence of expression of EZH2 comprises detecting the presence of EZH2 mRNA.

29. The method of Claim 28, wherein said detecting the presence of expression of hepsin mRNA comprises exposing said hepsin mRNA to a nucleic acid probe complementary to said hepsin mRNA.

30. The method of Claim 27, wherein said detecting the presence of expression of EZH2 comprises detecting the presence of a EZH2 polypeptide.

31. The method of Claim 30, wherein said detecting the presence of a EZH2 polypeptide comprises exposing said EZH2 polypeptide to an antibody specific to said EZH2 polypeptide and detecting the binding of said antibody to said EZH2 polypeptide.

32. The method of Claim 27, wherein said subject comprises a human subject.

33. The method of Claim 27, wherein said sample comprises tumor tissue.

34. The method of Claim 27, wherein said characterizing said prostate tissue comprises identifying a stage of prostate cancer in said prostate tissue.

35. The method of Claim 34, wherein said stage is selected from the group consisting of high-grade prostatic intraepithelial neoplasia, benign prostatic hyperplasia, prostate carcinoma, and metastatic prostate carcinoma.

36. The method of Claim 27, further comprising the step of c) providing a prognosis to said subject.

37. The method of Claim 36, wherein said prognosis comprises a risk of developing metastatic prostate cancer.

38. A kit for characterizing prostate cancer in a subject, comprising:

a) a reagent capable of specifically detecting the presence of absence of expression of hepsin; and

d) instructions for using said kit for characterizing cancer in said subject.

39. The kit of Claim 38, wherein said reagent comprises a nucleic acid probe complementary to a hepsin mRNA.

40. The kit of Claim 38, wherein said reagent comprises an antibody that specifically binds to a hepsin polypeptide.

41. The kit of Claim 38, wherein said instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

42. A kit for characterizing prostate cancer in a subject, comprising:

a) a reagent capable of specifically detecting the presence of absence of expression of pim-1; and

d) instructions for using said kit for characterizing cancer in said subject.

43. The kit of Claim 42, wherein said reagent comprises a nucleic acid probe complementary to a pim-1 mRNA.

44. The kit of Claim 42, wherein said reagent comprises an antibody that specifically binds to a pim-1 polypeptide.

45. The kit of Claim 42, wherein said instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

46. A kit for characterizing prostate cancer in a subject, comprising:

a) a reagent capable of specifically detecting the presence of absence of expression of EZH2; and

d) instructions for using said kit for characterizing cancer in said subject.

47. The kit of Claim 46, wherein said reagent comprises a nucleic acid probe complementary to a EZH2 mRNA.

48. The kit of Claim 46, wherein said reagent comprises an antibody that specifically binds to a EZH2 polypeptide.

49. The kit of Claim 46, wherein said instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

50. A method of screening compounds, comprising:

a) providing

i) a prostate cell sample; and

ii) one or more test compounds; and

b) contacting said prostate cell sample with said test compound; and

c) detecting a change in hepsin expression in said prostate cell sample in the presence of said test compound relative to the absence of said test compound.

51. The method of Claim 50, wherein said detecting comprises detecting hepsin mRNA.

52. The method of Claim 50, wherein said detecting comprises detecting hepsin polypeptide.

53. The method of Claim 50, wherein said cell is *in vitro*.

54. The method of Claim 50, wherein said cell is *in vivo*.

55. The method of Claim 50, wherein said test compound comprises an antisense compound.

56. The method of Claim 50, wherein said test compound comprises a drug.

57. A method of screening compounds, comprising:

a) providing

i) a prostate cell sample; and

ii) one or more test compounds; and

b) contacting said prostate cell sample with said test compound; and

c) detecting a change in pim-1 expression in said prostate cell sample in the presence of said test compound relative to the absence of said test compound.

58. The method of Claim 57, wherein said detecting comprises detecting pim-1 mRNA.

59. The method of Claim 57, wherein said detecting comprises detecting pim-1 polypeptide.

60. The method of Claim 57, wherein said cell is *in vitro*.

61. The method of Claim 57, wherein said cell is *in vivo*.

62. The method of Claim 57, wherein said test compound comprises an antisense compound.

63. The method of Claim 57, wherein said test compound comprises a drug.

64. A method of screening compounds, comprising:

a) providing

i) a prostate cell sample; and

ii) one or more test compounds; and

b) contacting said prostate cell sample with said test compound; and

c) detecting a change in EZH2 expression in said prostate cell sample in the presence of said test compound relative to the absence of said test compound.

65. The method of Claim 64, wherein said detecting comprises detecting EZH2 mRNA.

66. The method of Claim 64, wherein said detecting comprises detecting EZH2 polypeptide.

67. The method of Claim 64, wherein said cell is *in vitro*.

68. The method of Claim 64, wherein said cell is *in vivo*.

69. The method of Claim 64, wherein said test compound comprises an antisense compound.

70. The method of Claim 64, wherein said test compound comprises a drug.

71. A method for characterizing inconclusive prostate biopsy tissue in a subject, comprising:

a) providing an inconclusive prostate biopsy tissue sample from a subject; and

b) detecting the presence of expression of AMACR in said sample, thereby characterizing said inconclusive prostate biopsy tissue sample.

72. The method of Claim 71, wherein said detecting the presence of expression of AMACR comprises detecting the presence of AMACR mRNA.

73. The method of Claim 72, wherein said detecting the presence of expression of AMACR mRNA comprises exposing said AMACR mRNA to a nucleic acid probe complementary to at least a portion of said AMACR mRNA.

74. The method of Claim 71, wherein said detecting the presence of expression of AMACR comprises detecting the presence of a AMACR polypeptide.

75. The method of Claim 74, wherein said detecting the presence of a AMACR polypeptide comprises exposing said AMACR polypeptide to an antibody specific to said AMACR polypeptide and detecting the binding of said antibody to said AMACR polypeptide.

76. The method of Claim 71, wherein said subject comprises a human subject.

77. The method of Claim 71, wherein the presence of AMACR expression in said inconclusive biopsy tissue is indicative of prostate cancer in said subject.

78. The method of Claim 71, wherein said method further comprises the step of detecting expression of a basal cell marker selected from the group consisting of 34βE12 and p63 and the absence of a basal cell marker expression and the presence of AMACR expression is indicative of prostate cancer in said subject.

79. A method of detecting AMACR expression in a bodily fluid, comprising:

a) providing

i) a bodily fluid from a subject; and

ii) a reagent for detecting AMACR expression in said biological fluid; and

b) contacting said bodily fluid with said reagent under conditions such that said reagent detects AMACR expression in said bodily fluid.

80. The method of claim 79, wherein said bodily fluid is selected from the group consisting of serum, urine, whole blood, lymph fluid, and mucus.

81. The method of claim 79, wherein the presence of AMACR in said bodily fluid is indicative of cancer.

82. The method of claim 79, wherein said cancer is prostate cancer.

83. A kit for characterizing inconclusive prostate biopsy tissue in a subject, comprising:

a) a reagent capable of specifically detecting the presence or absence of expression of AMACR; and

b) instructions for using said kit for characterizing inconclusive biopsy tissue in said subject.

84. The kit of Claim 83, wherein said reagent comprises a nucleic acid probe complementary to at least a portion of an AMACR mRNA.

85. The kit of Claim 83, wherein said reagent comprises an antibody that specifically binds to a AMACR polypeptide.

86. The kit of Claim 83, wherein said kit further comprises a second reagent, said second reagent capable of specifically detecting the expression of a basal cell marker selected from the group consisting of 34βE12 and p63.

87. The kit of claim 86, wherein said instructions further comprise instructions for using said second reagent and said reagent for characterizing inconclusive biopsy tissue in said subject.

88. The kit of Claim 83, wherein said instructions comprise instructions required by the United States Food and Drug Administration for use in *in vitro* diagnostic products.

89. A method of characterizing tissue in a subject, comprising:

a) providing a tissue sample from a subject, said tissue sample selected from the group consisting of breast tissue, ovarian tissue, lymph tissue, and melanoma tissue; and

b) detecting the presence or absence of expression of AMACR in said sample, thereby characterizing said breast tissue sample.

90. A method of diagnosing cancer in a subject, comprising:

a) providing a tissue sample from a subject, said tissue sample selected from the group consisting of breast tissue, ovarian tissue, lymph tissue, and melanoma tissue; and wherein said subject is suspected of having breast cancer; and

b) detecting the presence of expression of AMACR in said sample, thereby diagnosing cancer in said subject.

91. A method of diagnosing cancer in a subject, comprising:

a) providing a blood sample from a subject suspected of having cancer; and

b) detecting an immune response to AMACR in said blood sample, thereby diagnosing cancer in said subject.

92. The method of claim 91, wherein said cancer is prostate cancer.

93. The method of claim 91, wherein said detecting an immune response comprises detecting an antibody against said AMACR in said blood sample.

94. A method of inhibiting the growth of cells, comprising

a) providing

i) a cell that expresses EZH2; and

i) a reagent for inhibiting EZH2 expression in said cell; and

b) contacting said cell with said reagent under conditions such that the expression of EZH2 in said cell is inhibited.

95. The method of claim 94, wherein said reagent is an antisense oligonucleotide.

96. The method of claim 94, wherein said reagent is a RNA duplex.

97. The method of claim 94, wherein said reagent is a drug.

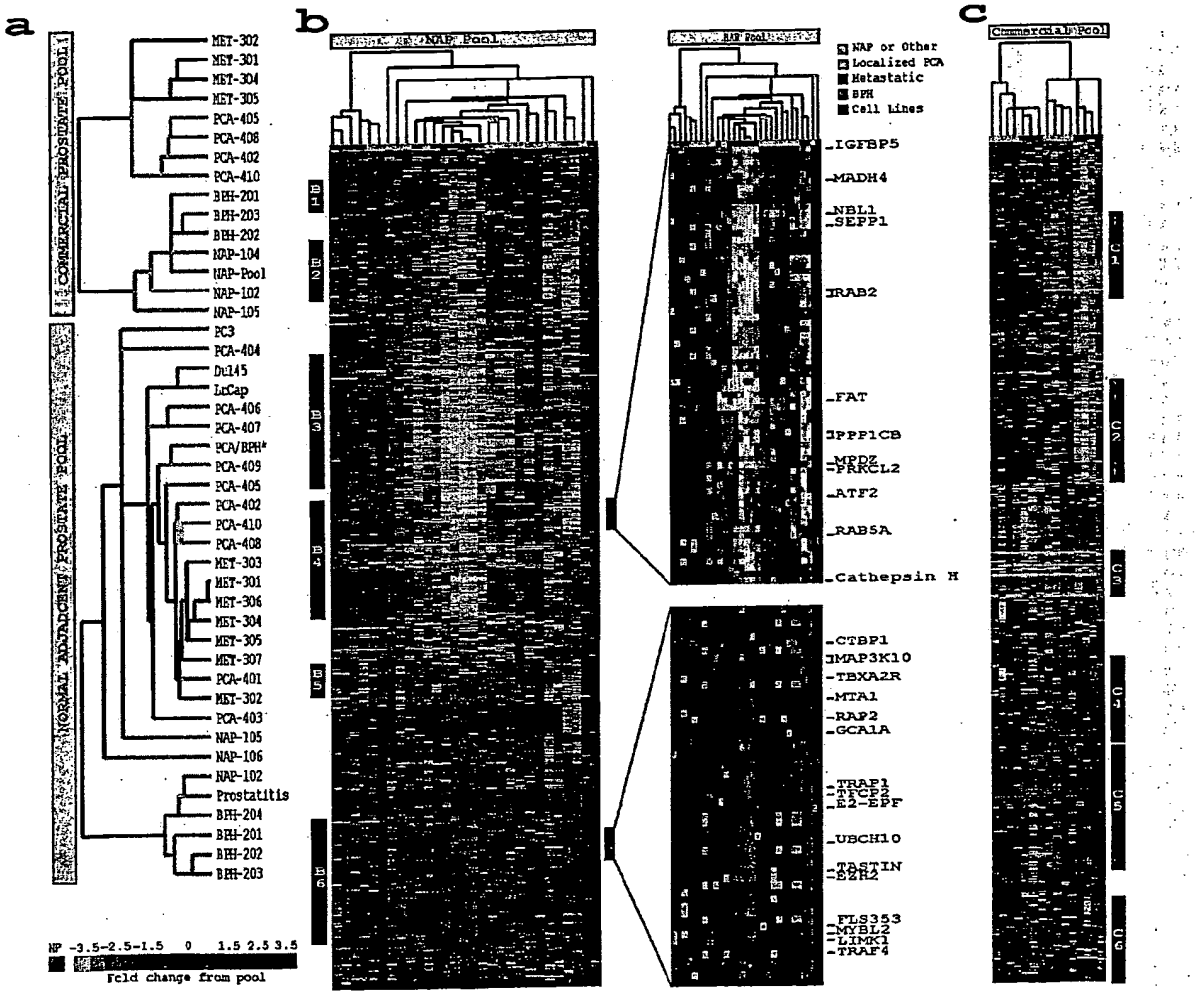
98. The method of claim 94, wherein said cell is a prostate cancer cell.

99. The method of claim 94, wherein said cell is *in vitro*.

100. The method of claim 94, wherein said cell is *in vivo*.

101. The method of claim 94, wherein said contacting further results in a decrease in proliferation of said cell.

Figure 1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b
c
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MET-301
MET-304
MET-305
PCA-405
PCA-408
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BPH-203
BPH-202
NAP-104
NAP-Pool
NAP-102
NAP-105
PC3
PCA-404
Du145
LnCap
PCA-406
PCA-407
PCA/BPH*
PCA-409
PCA-405
PCA-402
PCA-410
PCA-408
MET-303
MET-301
MET-306
MET-304
MET-305
MET-307
PCA-401
MET-302
PCA-403
NAP-105
NAP-106
NAP-102
Prostatitis
BPH-204
BPH-201
BPH-202
BPH-203
NAP or Other
Localized PCA
Metastatic
BPH
Cell Lines
IGFBP5
MADH4
NBL1
SEPP1
IRAB2
FAT
IPPF1CB
MPDZ
PRRCL2
ATF2
RAB5A
Cathepsin H
CTBP1
MAP3K10
TBXA2R
MTA1
RAP2
GCA1A
TRAP1
TTCP2
E2-EPF
UBCH10
TASTIN
EZH2
FLS353
MYBL2
LIMK1
TRAF4
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Fold change from pool

Figure 2

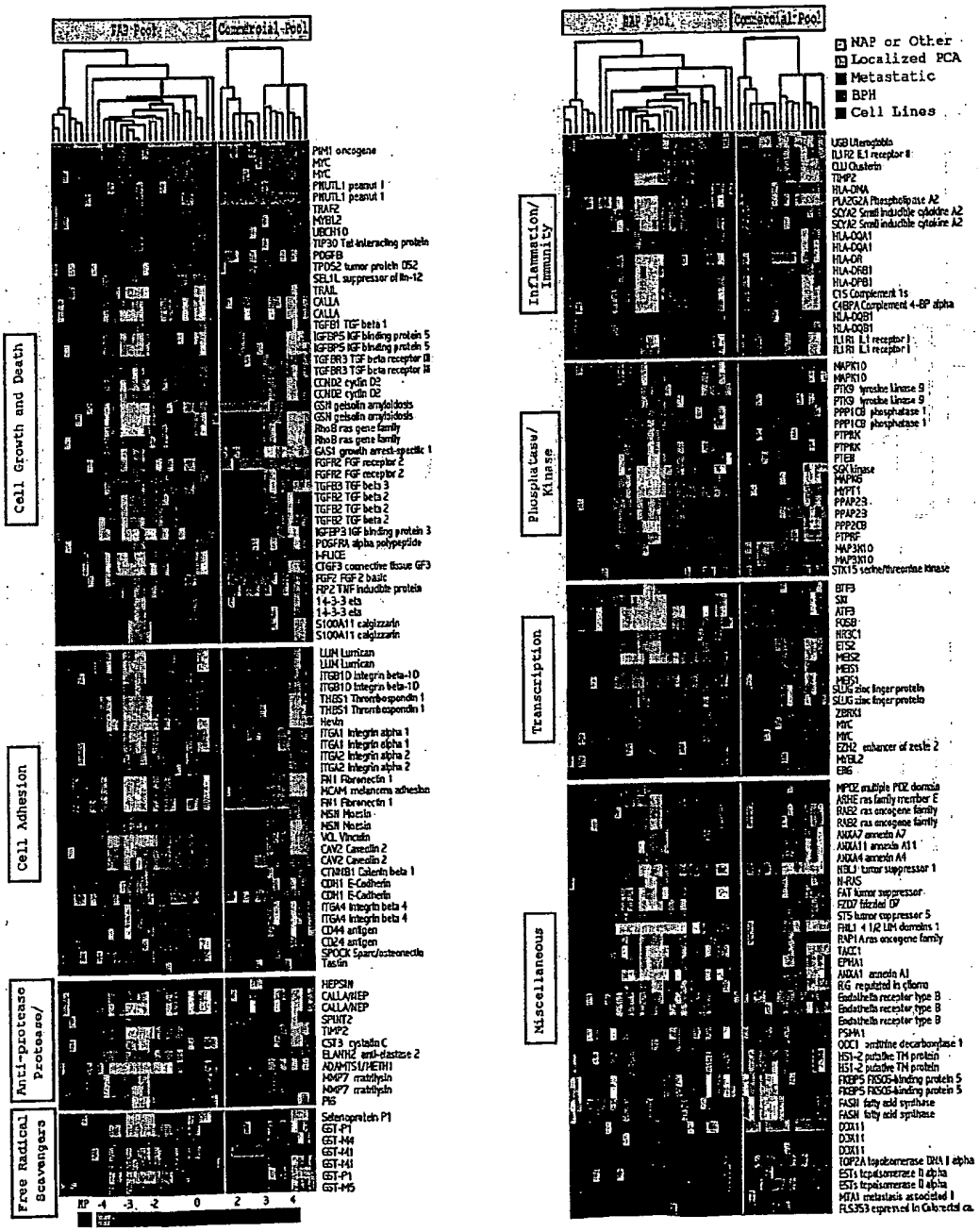

NAP Pool
Commercial Pool
NAP or Other
Localized PCA
Metastatic
BPH
Cell Lines
Cell Growth and Death
Cell Adhesion
Anti-protease Protease/
Free Radical Scavengers
Inflammation/ Immunity
Phospha tase/ Kinase
Transcription
Miscellaneous
PIM1 oncogene
MYC
MYC
PRUTL1 peanut 1
PRUTL1 peanut 1
TRAF2
MYBL2
UBCH10
TIP30 Tat-interacting protein
PDGFB
TP053 tumor protein 053
SEL1L suppressor of lin-12
TRAIL
CALLA
CALLA
TGFB1 TGF beta 1
IGFBP5 IGF binding protein 5
IGFBP5 IGF binding protein 5
TGFBR3 TGF beta receptor III
TGFBR3 TGF beta receptor III
CCND2 cyclin D2
CCND2 cyclin D2
GSN gelsolin amyloidosis
GSN gelsolin amyloidosis
Rho B ras gene family
Rho B ras gene family
GAS1 growth arrest-specific 1
FGFR2 FGF receptor 2
FGFR2 FGF receptor 2
TGFB3 TGF beta 3
TGFB2 TGF beta 2
TGFB2 TGF beta 2
TGFB2 TGF beta 2
IGFBP3 IGF binding protein 3
PDGFRA alpha polypeptide
M-RLICE
CTGF3 connective tissue GF3
FGF2 FGF 2 basic
RP2 TNF inducible protein
14-3-3 eta
14-3-3 eta
S100A11 calgizzarin
S100A11 calgizzarin
LUM Lumican
LUM Lumican
ITGB1D integrin beta-1D
ITGB1D integrin beta-1D
THBS1 Thrombospondin 1
THBS1 Thrombospondin 1
Hevin
ITGA1 Integrin alpha 1
ITGA1 Integrin alpha 1
ITGA2 Integrin alpha 2
ITGA2 Integrin alpha 2
FN1 Fibronectin 1
MCAM melanoma adhesion
FN1 Fibronectin 1
MSN Moesin
MSN Moesin
VCL Vinculin
CAV2 Caveolin 2
CAV2 Caveolin 2
CTNNB1 Catenin beta 1
CDH1 E-Cadherin
CDH1 E-Cadherin
ITGA4 Integrin beta 4
ITGA4 Integrin beta 4
CD44 antigen
CD24 antigen
SPOCK Sparc/osteonectin
Testin
HEPSIN
CALLA/NEP
CALLA/NEP
SPINT2
TIMP2
CST3 cystatin C
ELANH2 anti-elastase 2
ADAMTS1/METH1
MMP7 matrilysin
MMP7 matrilysin
PI6
Selenoprotein P1
GST-P1
GST-M4
GST-M1
GST-M1
GST-P1
GST-M5
RP -4 -3 -2 0 2 3 4
UGB Uteroglobin
IL1R2 IL1 receptor II
CLU Clusterin
TIMP2
HLA-DMA
PLA2G2A Phospholipase A2
SCYA2 Small inducible cytokine A2
SCYA2 Small inducible cytokine A2
HLA-DQA1
HLA-DQA1
HLA-DR
HLA-DRB1
HLA-DPB1
C1S Complement 1s
C4BPA Complement 4-BP alpha
HLA-DQB1
HLA-DQB1
IL1R1 IL1 receptor I
IL1R1 IL1 receptor I
MAPK10
MAPK10
PTK9 tyrosine kinase 9
PTK9 tyrosine kinase 9
PPP1CB phosphatase 1
PPP1CB phosphatase 1
PTPRK
PTPRK
PTEN
SGK kinase
MAPK6
MPT1
PPAP2B
PPAP2B
PPP2CB
PTPRF
MAP3K10
MAP3K10
STK15 serine/threonine kinase
BTF3
SKI
ATF3
RGS3
NR3C1
ETS2
MEIS2
MEIS1
MEIS1
SUG zinc finger protein
SUG zinc finger protein
ZBRK1
MYC
MYC
EZH2 enhancer of zeste 2
MYBL2
EB6
MPDZ multiple PDZ domain
ARHE ras family member E
RAB2 ras oncogene family
RAB2 ras oncogene family
ANXA7 annexin A7
ANXA11 annexin A11
ANXA4 annexin A4
NBL1 tumor suppressor 1
N-RAS
FAT tumor suppressor
FZD7 frizzled 7P
ST5 tumor suppressor 5
FHL1 4 1/2 LIM domains 1
RAP1A ras oncogene family
TACC1
EPHA1
ANXA1 annexin A1
RG regulated in glioma
Endothelin receptor type B
Endothelin receptor type B
Endothelin receptor type B
PSPH1
ODC1 ornithine decarboxylase 1
HS1-2 putative TM protein
HS1-2 putative TM protein
FREP5 FKSG5-binding protein 5
FREP5 FKSG5-binding protein 5
FASN fatty acid synthase
FASN fatty acid synthase
DDX11
DDX11
DDX11
TOP2A topoisomerase DNA II alpha
ESTs topoisomerase II alpha
ESTs topoisomerase II alpha
MTA1 metastasis associated 1
FLS353 expressed in Colorectal ca.

Figure 3

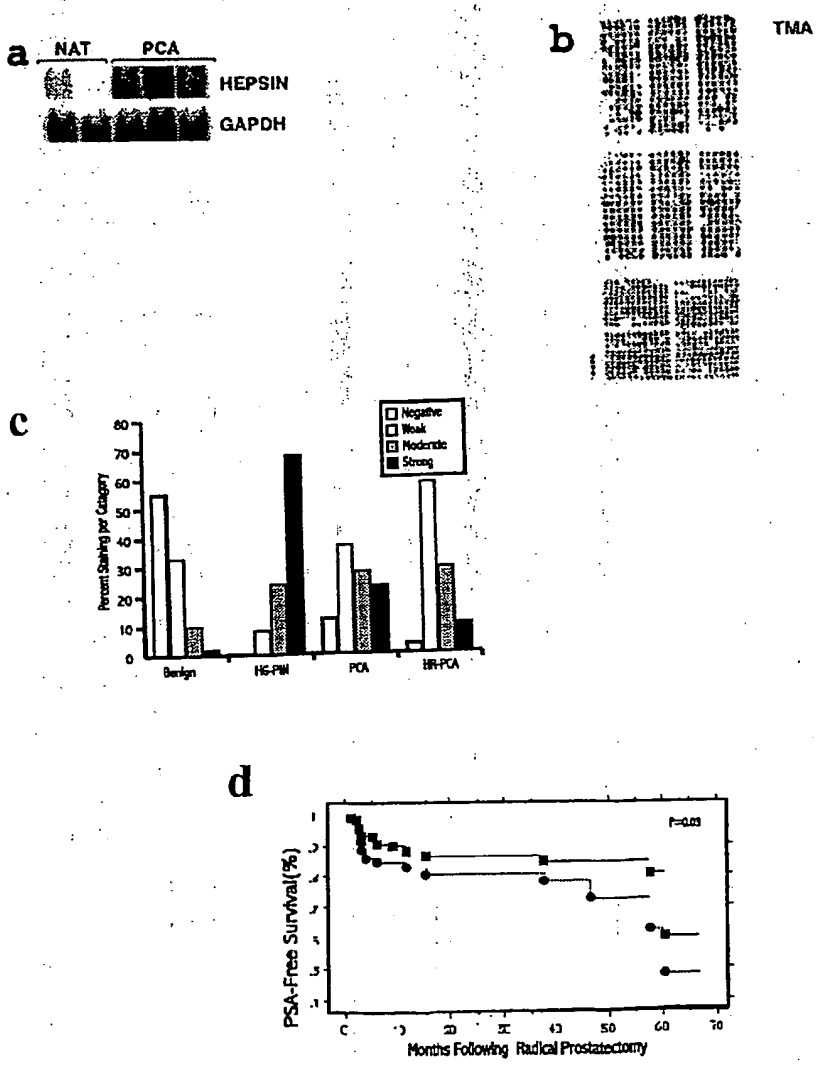
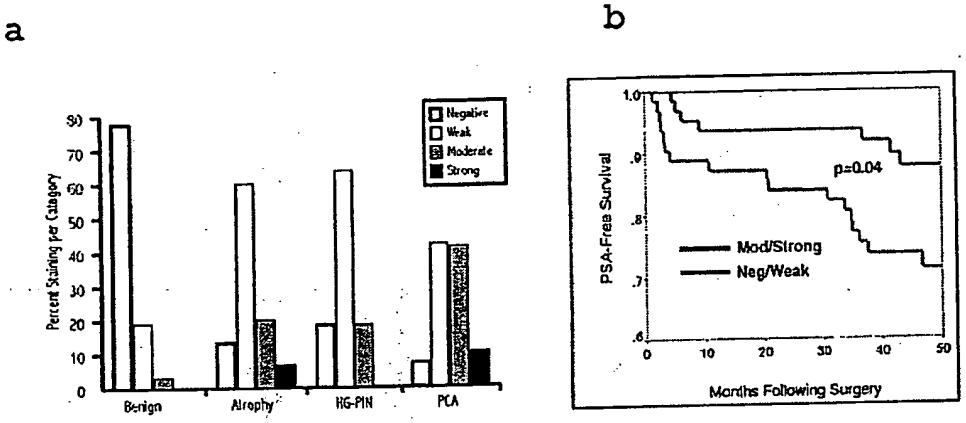

a
NAT
PCA
HEPSIN
GAPDH
b
TMA
c
Negative
Weak
Moderate
Strong
Percent Staining per Category
Benign
HG-PIN
PCA
HR-PCA
d
PSA-Free Survival(%)
Months Following Radical Prostatectomy

Figure 4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b
Negative
Weak
Moderate
Strong
Percent Staining per Category
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Atrophy
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PCA
PSA-Free Survival
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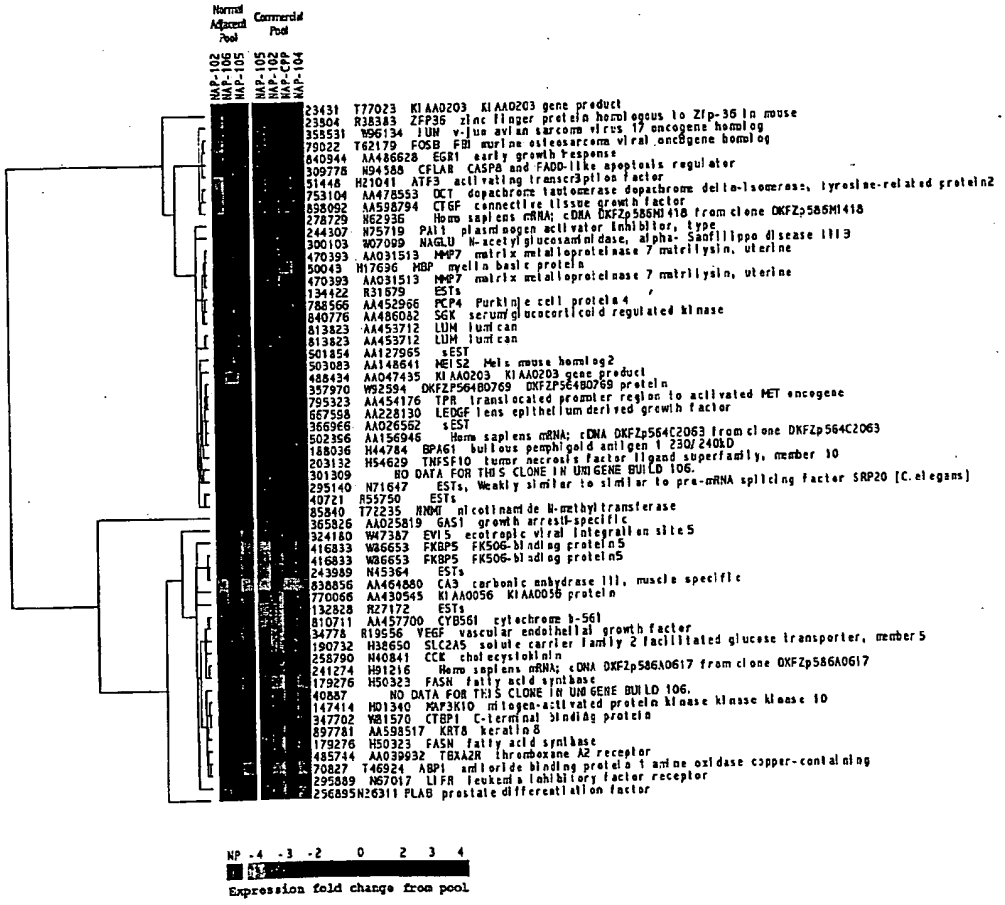
Mod/Strong
Neg/Weak
Months Following Surgery

Figure 5


Normal Adjacent Pool
Commercial Pool
MAP-102
MAP-103
MAP-105
MAP-105
MAP-CPP
MAP-104
23431 T77023 KIAA0203 KIAA0203 gene product
23504 R38383 ZFP36 zinc finger protein homologous to Zfp-36 in mouse
355531 W96134 JUN v-jun avian sarcoma virus 17 oncogene homolog
79022 T62179 FOSB FBJ murine osteosarcoma viral oncogene homolog
840944 AA486628 EGR1 early growth response
309778 H94588 CFLAR CASP8 and FADD-like apoptosis regulator
51448 H21041 ATF3 activating transcription factor
753104 AA478553 DCT dopachrome tautomerase dopachrome delta-isomerase, tyrosine-related protein2
898092 AA598794 CTGF connective tissue growth factor
278729 H62936 Homo sapiens mRNA; cDNA DKFZp586M1418 from clone DKFZp586M1418
244307 N75719 PAI1 plasminogen activator inhibitor, type
300103 W07099 NAGLU N-acetylglucosaminidase, alpha- Saofilippo disease IIIB
470393 AA031513 MMP7 matrix metalloproteinase 7 matrilysin, uterine
50043 H17696 MBP myelin basic protein
470393 AA031513 MMP7 matrix metalloproteinase 7 matrilysin, uterine
134422 R31679 ESTs
788566 AA452966 PCP4 Purkinje cell protein 4
840776 AA486082 SGK serum/glucocorticoid regulated kinase
813823 AA453712 LUM lumican
813823 AA453712 LUM lumican
501854 AA127965 sEST
503083 AA148641 MEIS2 Meis mouse homolog2
488434 AA047435 KIAA0203 KIAA0203 gene product
357970 W92594 DKFZP564B0769 DKFZP564B0769 protein
795323 AA454176 TPR translocated promoter region to activated MET oncogene
667598 AA228130 LEDGF lens epithelium derived growth factor
366966 AA026562 sEST
502356 AA156946 Homo sapiens mRNA; cDNA DKFZp564C2063 from clone DKFZp564C2063
188036 H44784 BPAG1 bullous pemphigoid antigen 1 230/240kD
203132 H54629 TNFSF10 tumor necrosis factor ligand superfamily, member 10
301309 NO DATA FOR THIS CLONE IN UNIGENE BUILD 106.
295140 N71647 ESTs, Weakly similar to similar to pre-mRNA splicing factor SRP20 [C.elegans]
40721 R55750 ESTs
85840 T72235 NNMT nicotinamide N-methyltransferase
365826 AA025819 GAS1 growth arrest-specific
324180 W47387 EVI5 ecotropic viral integration site 5
416833 W86653 FKBP5 FK506-binding protein5
416833 W86653 FKBP5 FK506-binding protein5
243989 N45364 ESTs
838856 AA464880 CA3 carbonic anhydrase III, muscle specific
770066 AA430545 KIAA0056 KIAA0056 protein
132828 R27172 ESTs
810711 AA457700 CYB561 cytochrome b-561
34778 R19556 VEGF vascular endothelial growth factor
190732 H38650 SLC2A5 solute carrier family 2 facilitated glucose transporter, member 5
258790 N40841 CCK cholecystokinin
241274 H91216 Homo sapiens mRNA; cDNA DKFZp586A0617 from clone DKFZp586A0617
179276 H50323 FASN fatty acid synthase
40887 NO DATA FOR THIS CLONE IN UNIGENE BUILD 106.
147414 H01340 MAP3K10 mitogen-activated protein kinase kinase kinase 10
347702 W81570 CTBP1 C-terminal binding protein
897781 AA598517 KRT8 keratin 8
179276 H50323 FASN fatty acid synthase
485744 AA039932 TBXA2R thromboxane A2 receptor
70827 T46924 ABP1 amiloride binding protein 1 amine oxidase copper-containing
295889 N67017 LIFR leukemia inhibitory factor receptor
256895N26311 PLAB prostate differentiation factor
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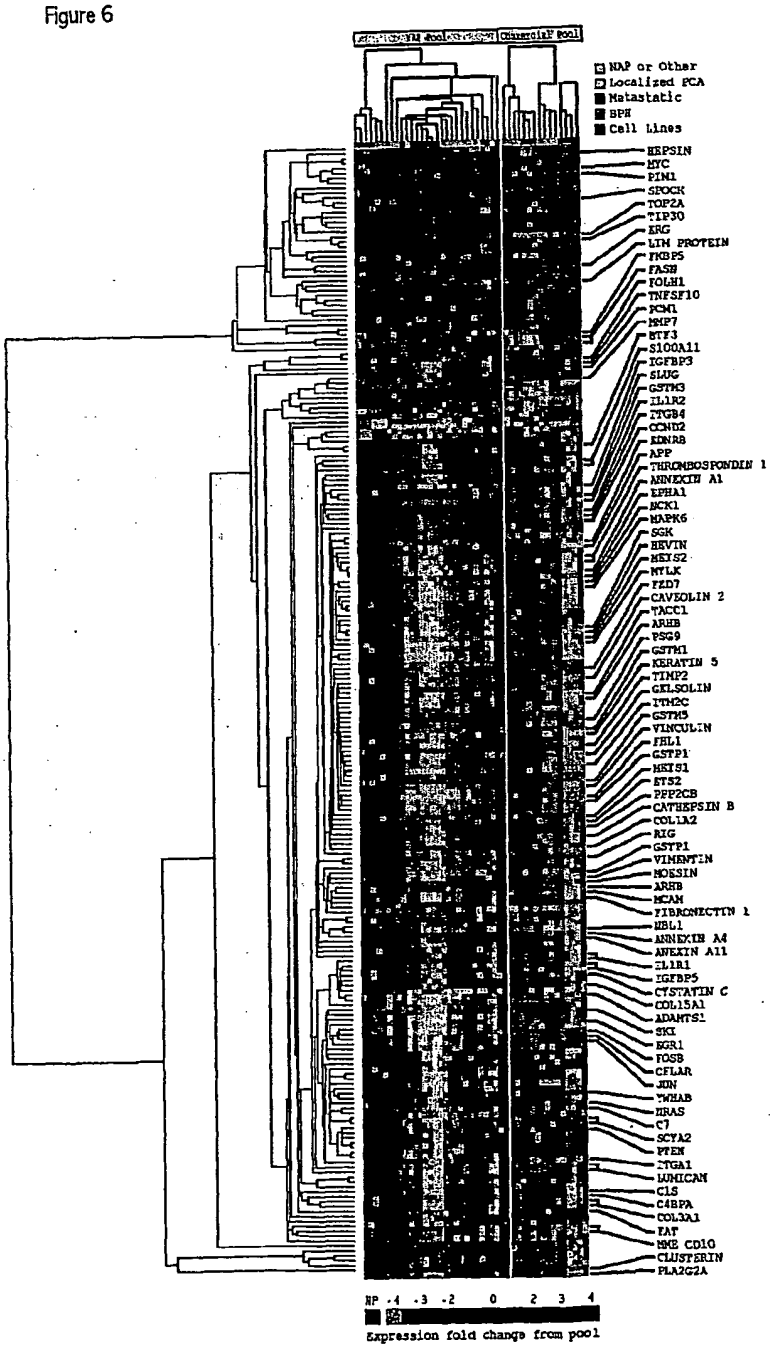
Expression fold change from pool

Figure 6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BPH
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MYC
PIM1
SPOCK
TOP2A
TIP30
ERG
LIN PROTEIN
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FASN
FOLH1
TNFSF10
PCM1
MMP7
STX3
S100A11
IGFBP3
SLUG
GSTM3
IL1R2
TGFB4
CCND2
EDNRB
APP
THROMBOSPONDIN 1
ANNEXIN A1
EPHA1
NCK1
MAPK6
SGK
HEVIN
MEIS2
MYLK
FED7
CAVEOLIN 2
TACC1
ARHB
PSG9
GSTM1
KERATIN 5
TIMP2
GELSOLIN
ITM2C
GSTM5
VINCULIN
FHL1
GSTP1
HKIS1
STS2
PPP2CB
CATHEPSIN B
COL1A2
RIG
GSTP1
VIMENTIN
MOESIN
ARHB
MCAM
FIBRONECTIN 1
MBL1
ANNEXIN A4
ANEXIN A11
IL1R1
IGFBP5
CYSTATIN C
COL15A1
ADAMTS1
SKI
EGR1
FOSB
CFLAR
JUN
YWHAB
HRAS
C7
SCYA2
PTEN
ITGA1
LUMICAN
C1S
C4BPA
COL3A1
TAF
MME CD10
CLUSTERIN
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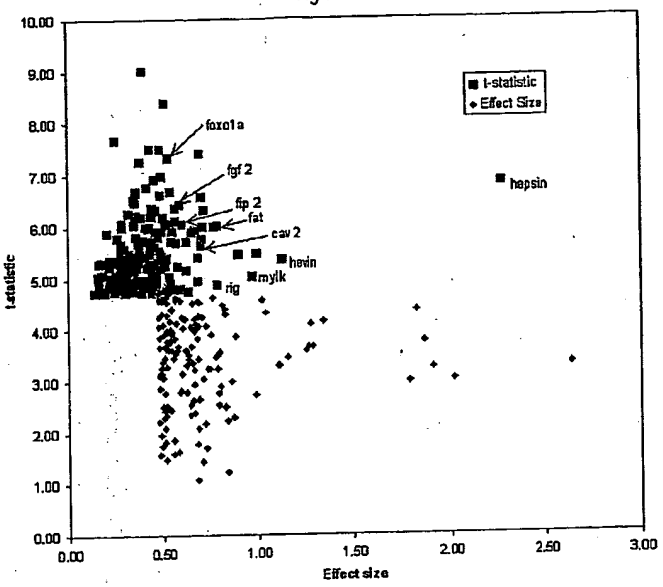
Expression fold change from pool

Figure 7

a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fgf 2
fip 2
fat
cav 2
hevin
mylk
rig
hepsin
t-statistic
Effect size
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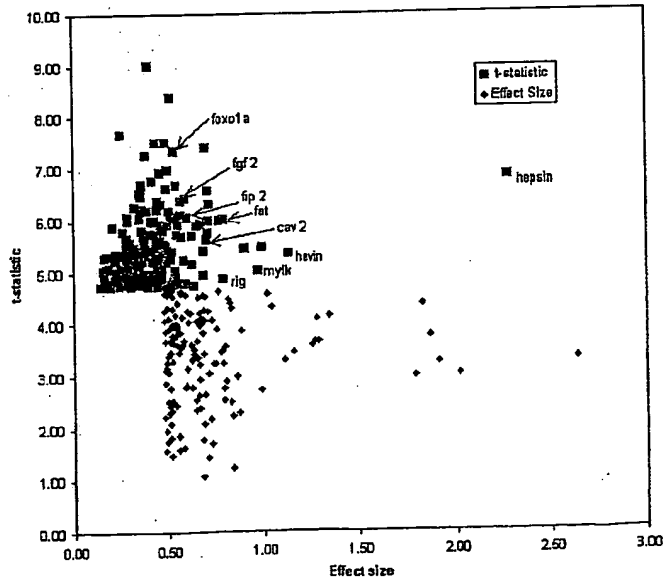
0.50
1.00
1.50
2.00
2.50
3.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00

b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Effect Size
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fgf 2
fip 2
fat
cav 2
hevin
mylk
rig
hepsin
t-statistic
Effect size
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0.50
1.00
1.50
2.00
2.50
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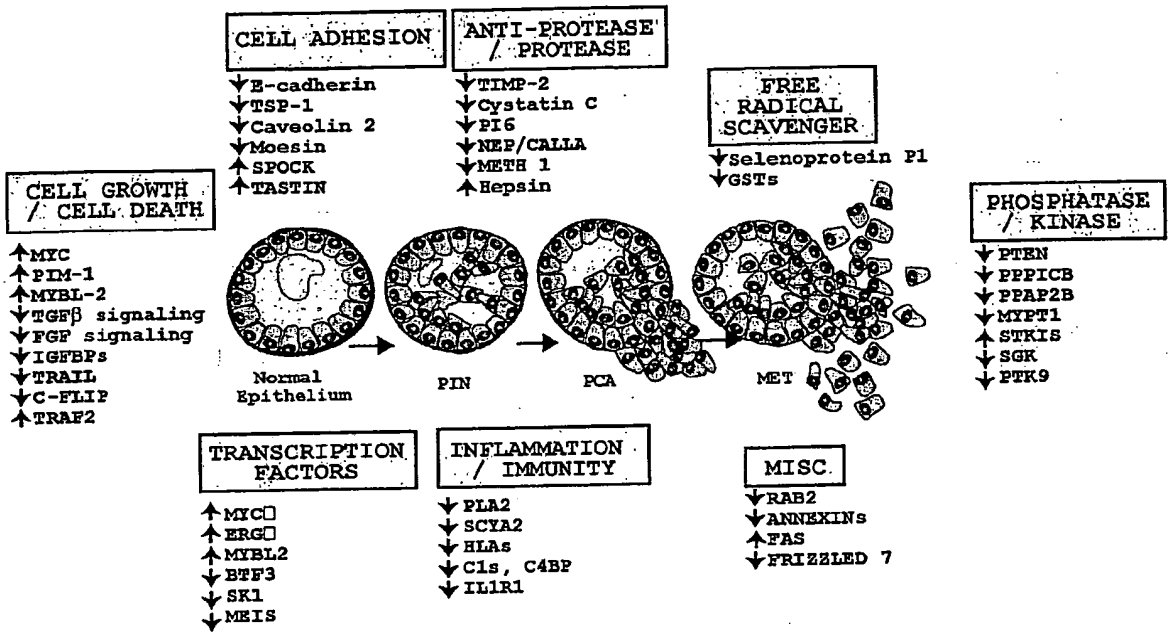
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00

Figure 8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E-cadherin
TSP-1
Caveolin 2
Moesin
SPOCK
TASTIN
ANTI-PROTEASE / PROTEASE
TIMP-2
Cystatin C
PI6
NEP/CALLA
METH 1
Hepsin
FREE RADICAL SCAVENGER
Selenoprotein P1
GSTs
CELL GROWTH / CELL DEATH
MYC
PIM-1
MYBL-2
TGFβ signaling
FGF signaling
IGFBPs
TRAIL
C-FLIP
TRAF2
PHOSPHATASE / KINASE
PTEN
PPPICB
PPAP2B
MYPT1
STKIS
SGK
PTK9
Normal Epithelium
PIN
PCA
MET
TRANSCRIPTION FACTORS
MYC
ERG
MYBL2
BTF3
SK1
MEIS
INFLAMMATION / IMMUNITY
PLA2
SCYA2
HLAs
C1s, C4BP
IL1R1
MISC.
RAB2
ANNEXINs
FAS
FRIZZLED 7

Figure 9

SEQ ID NO	Name	Accession Number
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4	FASN	NM_004104
5	FOLH1	M99487
6	TNFSF10	XM_045049
7	PCM1	XM_044711
8	S100A11	XM_047223
9	IGFBP3	XM_004689
10	SLUG	XM_011634
11	GSTM3	J05459
12	IL1R2	X59770
13	ITGB4	X53587
14	CCND2	XM_034568
15	EDNRB	S57283
16	APP	X06989
17	THROMBOSPONDIN 1	X04665
18	ANNEXIN A1	XM_005665
19	EPHA1	M18391
20	NCK1	XM_051968
21	MAPK6	XM_017662
22	SGK	XM_037045
23	HEVIN	XM_011533
24	MEIS2	XM_012430
25	MYLK	XM_042191
26	FZD7	NM_003507
27	CAVEOLIN 2	XM_004966
28	TACC1	XM_049505
29	ARHB	XM_002689
30	PSG9	NM_002784
31	GSTM1	NM_000561
32	Keratin 5	XM_006847
33	TIMP2	XM_027036
34	GELSOLIN	XM_016545
35	ITM2C	AA034213
36	GSTM5	XM_002154
37	VINCULIN	XM_011883
38	FHL1	XM_042931

Figure 9 (cont.)

39	GSTP1	XM_040116
40	MEIS1	XM_010880
41	ETS2	XM_009766
42	PPP2CB	XM_005121
43	CATHEPSIN B	XM_005133
44	COL1A2	XM_029246
45	RIG	XM_006029
46	VIMENTIN	XM_042952
47	MOESIN	XM_013042
48	MCAM	XM_006077
49	FIBRONECTIN 1	XM_030549
50	NBL1	XM_001434
51	ANNEXIN A4	XM_031594
52	ANNEXIN A11	XM_035906
53	IL1R1	XM_002686
54	IGFBP5	XM_046731
55	CYSTATIN C	XM_009599
56	COL15A1	XM_005592
57	ADAMTS1	XM_047796
58	SKI	XM_001535
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62	JUN	XM_001472
63	YWHAB	XM_009519
64	NRAS	XM_001317
65	C7	J03507
66	SCYA2	XM_038982
67	ITGA1	XM_032902
68	LUMICAN	XM_006900
69	C1S	XM_032536
70	C4BPA	XM_052053
71	COL3A1	XM_044878
72	FAT	XM_003477
73	MMECD10	XM_030168
74	CLUSTERIN	XM_005113
75	PLA2G2A	XM_027887
76	MADH4	XM_030100
77	SEPP1	XM_011306
78	RAB2	XM_037653
79	PP1CB	NM_002709
80	MPDZ	XM_051281

Figure 9 (cont.)

81	PRKCL2	XM_001880
82	ATF2	XM_027217
83	RAB5A	NM_004162
84	Cathepsin H	XM_007633
85	CTBP1	XM_003445
86	MAP3K10	XM_042665
87	TBXA2R	XM_047633
88	MTA1	NM_004689
89	RAP2	NM_002886
90	TRAP1	XM_036666
91	TFCP2	XM_051171
92	E2-EPF	XM_012615
93	UBCH10	XM_009488
94	TASTIN	XM_006826
95	EZH2	XM_004774; NM004456
96	FLS353	AB024704
97	MYBL2	XM_009492
98	LIMK1	XM_051836
99	TRAF4	XM_031428
104	AMACR	XM_043772; NM01324
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115	CTBP2	AF016507
116	Annexin A2	NM_004039
117	Annexin A4	XM_031596
118	Annexin A11	NM_001157
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121	TOP2A	NM_001067
122	VaV2	XM_005638

Figure 10

SEQ ID NO:1

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661   attgccccag aggccgtttc ttggccgcca tctgccaaga ctgtggccgc aggaagctgc
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SEQ ID NO:2

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Figure 10 (cont.)

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1261  tccacagcct gtcgccgggg cccagcaaat agcagccttt ctggcaggtc ctccctctc
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541   cactgtgggc gaaggagaag accacgacat tccaattgga attgacaaag ctctggagaa
601   aatgcagcgg gaagaacaat gtattttata tcttggacca agatatggtt ttggagaggc
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Figure 10 (cont.)

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SEQ ID NO:4

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301   cgaggaacac acactggcgt ctgggtgggc gtgagcggct ctgagacctc ggaggccctg
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421   gccaaccggc tctccttctt cttcgacttc agagggccca gcatcgcact ggacacagcc
481   tgctcctcca gcctgatggc cctgcagaac gcctaccagg ccatccacag cgggcagtgc
541   cctgccgcca tcgtgggggg catcaacgtc ctgctgaagc ccaacacctc cgtgcagttc
601   ttgaggctgg ggatgctcag ccccgagggc acctgcaagg ccttcgacac agcggggaat
661   gggtactgcc gctcggaggg tgtggtggct gtcctgctga ccaagaagtc cctggcccgg
721   aaggtctaca ccaccatcct gaacaaaggc accaatacag atggcttcaa ggagcaaggc
781   gtgaccttcc ctcaggatat ccaggagcag cctatccgct cgttgtacca gtcggccgga
841   gtggcccctg agtcatttga atacatcgaa gcccacggac caggcaccaa ggtgggcgac
901   ccccaggagc gtaatggcat cacccgagcc ctgtgcgcca cccgccagga gccgctgctc
961   atcggctcca ccaagtccaa catggggcac ccggagccag cctcggggct cgacgccctg
1021  gccaaggtgc tgctgtccct ggagcacggg ctctgggccc ccaacctgca cttccatagc
1081  cccaaccctg agatcccagc gctgttggat gggcggctgc aggtggtgga ccagcccctg
1141  cccgtccgtg gcggcaacgt gggcatcaac tcctttggct tcgggggctc caacatgcac
1201  atcatcctga ggcccaacac gcagtccgcc cccgcacccg ccccacatgc caccctgccc
1261  cgtctgctgc gggccagcgg acgcacccct gaggccgtgc agaagctgct ggagcagggc
1321  ctccggcaca gccagggcct ggctttcctg agcatgctga acgacatcgc ggctgtcccc
1381  gccaccgcca tgcccttccg tggctacgct gtgctgggtg gtgagacgcg gtggcccaga
1441  gtgcagcagg tgcccgctgg cgagcgcccg ctctggttca tctgctctgg gatgggcaca
1501  cagtggcgtg gaatggggct gagccttatg cgcctggacc gcttccgaga ttccatccta
1561  cgctccgatg aggctgtgaa ccgattcggc ctgaaggtgt cacagctgct gctgagcaca
1621  gacgagagca cctttgatga catcgtccat tcgtttgtga gcctgactgc catccagata
1681  ggcctcatag acctgctgag ctgcatggga cctgaggcag atggcatcgt cggccactcc
1741  ctgggggagt ggctgtcggt acgcgacggc tgcctgtccc aggaggaggc cgtcctcgct
1801  gcctactgga ggggacagtg catcaaagaa gccccacttc ccgccggcgc catggcagcc
1861  gtgggcttgt cctgggagga gtgtaaacag cgctgccccc ctgcggtggt gcccgcctgc
1921  cacaactcca aggacacagt caccatctcg ggacctcagg ccccggtgtt tgagttcgtg
1981  gagcagctga ggaaggaggg tgtgtttgcc aaggaggtgc ggaccggcgg tatggccttc
2041  cactcctact tcatggaggc catcgcaccc ccactgctgc aggagctcaa gaaggtgatc
2101  cgggagccga agccacgttc agcccgctgg ctcagcacct ctatccccga ggcccagtgg
```

Figure 10 (cont.)

```
2161 cacagcagcc tggcacgcac gtcttccgcc gagtacaatg tcaacaacct ggtgagccct
2221 gtgctgttcc aggaggccct gtggcacgtg cctgagcacg cggtggtgct ggagatcgcc
2281 ccgaccccgt gccctcaggc tgtcctgaag cgggtccgta agccgagctg caccatcatc
2341 ccccgtatga agaaggatca cagggacaac ctggagttct tcctggccgg catcggcagg
2401 ctgcacctct caggcatcga cgccaacccc aatgccttgt tcccacctgt ggagtcccca
2461 gctccccgag gaactccccc catctcccca ctcatcaagt gggaccacag cctggcctgg
2521 gacgcgccgg ccgccgagga cttccccaac ggttcaggtt ccccctcagc caccatctac
2581 acatgcacac caagctccga gtctcctgac cgctacctgg tggaccacac catcgacggt
2641 cgcgtcctct tccccgccac tggctacctg agcatagtgt ggaagacgct ggcccgcgcc
2701 tgggctgggc tcgagcagct gcctgtggtg tttgaggatg tggtgcagca ccaggccacc
2761 atcctgccca agactgggac agtgtccttg gaggtacggc tcctggaggc caccggtgcc
2821 ttcgaggtgt cagagaacgg caacctggta gtgagtggga aggtgtacca gtgggatgac
2881 cctgacccca ggctcttcga ccacccggaa agtccccacc ccaattcccc acggagtccc
2941 ctcttcctgg cccaggcaga agtttacaag gagctgcgtc tgcgtggcta cgactacggc
3001 cctcatttcc agggcatcct ggaggccagc ctggaaggtg actcggggag gctgctgtgg
3061 aaggataact gggtgagctt catggacacc atgctgcaga tgtccatcct gggctcggcc
3121 aagcacggcc tgtacctacc cacccgtgtc accgccatcc acatcgaccc tgccacccac
3181 aggcagaagc tgtacacact gcaggacaag gcccaagtgg ctgacgtggt ggtgagcagg
3241 tggccgaggg tcacagtggc gggaggcgtc cacatctccg ggctccacac tgagtcggcc
3301 ccgcggcggc acgaggagca gcaggtgccc atcctggaga agttttgctt cactccccac
3361 acggaggagg ggtgcctgtc tgagcacgct gccctcgagg aggagctgca actgtgcaag
3421 gggctggtcg aggcactcga gaccaaggtg acccagcagg ggctgaagat ggtggtgccg
3481 gactggacgg ggcccagatc cccccgggac ccctcacagc aggaactgcc ccggctgttg
3541 tcggctgcct gcaggcttca gctcaacggg aacctgcagc tggagctggc gcaggtgctg
3601 gcccaggaga ggcccaagct gccagaggac cctctgctca gcggcctcct ggactccccg
3661 gcactcaagg cctgcctgga cactgccgtg gagaacatgc ccagcctgaa gatgaaggtg
3721 gtggaggtgc tggccggcca cggtcacctg tattcccgca tcccaggcct gctcagcccc
3781 catcccctgc tgcagctgag ctacacggcc accgaccgcc acccccaggc cctggaggct
3841 gcccaggccg agctgcagca gcacgacgtt gcccagggcc agtgggatcc cgcagaccct
3901 gcccccagcg ccctgggcag cgcggacctc ctggtgtgca actgtgctgt ggctgccctc
3961 ggggacccgg cctcagctct cagcaacatg gtggctgccc tgagagaagg gggctttctg
4021 ctcctgcaca cactgctccg ggggcaccct cgggacatcg tggccttcct cacctccact
4081 gagccgcagt atggccaggg catcctgagc caggacgcgt gggagagcct cttctccagg
4141 gtgtcgctgc gcctggtggg cctgaagaag tccttctacg gcgccacgct cttcctgtgc
4201 cgccggccca ccccgcagga cagccccatc ttcctgccgg tggacgatac cagcttccgc
4261 tgggtggagt ctctgaaggg catcctggct gacgaagact cttcccggcc tgtgtggctg
4321 aaggccatca actgtgccac ctcgggcgtg gtgggcttgg tgaactgtct ccgccgagag
4381 cccggcggaa ccgtccggtg tgtgctgctc tccaacctca gcagcacctc ccacgtcccg
4441 gaggtggacc cgggctccgc agaactgcag aaggtgttgc agggagacct ggtgatgaac
4501 gtctaccgcg acggggcctg gggggttttc cgccacttcc tgctggagga caagcctgag
4561 gagccgacgg cacatgcctt tgtgagcacc ctcacccggg gggacctgtc ctccatccgc
4621 tgggtctgct cctcgctgcg ccatgcccag cccacctgcc ctggcgccca gctctgcacg
4681 gtctactacg cctccctcaa cttccgcgac atcatgctgg ccactggcaa gctgtccctt
4741 gatgccatcc cagggaagtg gacctcccag gacagcctgc taggtatgga gttctcgggc
4801 cgagacgcca gcggcaagcg tgtgatggga ctggtgcctg ccaagggcct ggccacctct
4861 gtcctgctgt caccggactt cctctgggat gtgccttcca actggacgct ggaggaggcg
4921 gcctcggtgc ctgtcgtcta cagcacggcc tactacgcgc tggtggtgcg tgggcgggtg
4981 cgccccgggg agacgctgct catccactcg ggctcgggcg gcgtgggcca ggccgccatc
5041 gccatcgccc tcagtctggg ctgccgcgtc ttcaccaccg tggggtcggc tgagaagcgg
5101 gcgtacctcc aggccaggtt cccccagctc gacagcacca gcttcgccaa ctcccgggac
5161 acatccttcg agcagcatgt gctgtggcac acgggcggga agggcgttga cctggtcttg
5221 aactccttgg cggaagagaa gctgcaggcc agcgtgaggt gcttcggtac gcacggtcgc
5281 ttcctggaaa ttggcaaatt cgacctttct cagaaccacc cgctcggcat ggctatcttc
5341 ctgaagaacg tgacattcca cggggtccta ctggatgcgt tcttcaacga gagcagtgct
```

Figure 10 (cont.)

```
5401  gactggcggg aggtgtgggc gcttgtcgag gccgccatcc gggatggggt ggtacggccc
5461  ctcaagtgca cggtgttcca tggggcccag gtggaggacg ccttccgcta catggcccaa
5521  gggaagcaca ttggcaaagt cgtcgtgcag gtgcttgcgg aggagccggc agtgctgaag
5581  ggggccaaac ccaagctgat gtcggccatc tccaagacct tctgcccggc ccacaagagc
5641  tacatcatcg ctggtggtct gggtggcttc ggcctggagt tggcgcagtg gctgatacag
5701  cgtggggtgc agaagctcgt gttgacttct cgctccggga tccggacagg ctaccaggcc
5761  aagcaggtcc gccggtggag gcgccagggg ctacaggtgc aggtgtccac cagcaacatc
5821  agctcactgg agggggcccg gggcctcatt gccgaggcgg cgcagcttgg gcccgtgggg
5881  ggcgtcttca acctggccgt ggtcttgaga gatggcttgc tggagaacca gaccccagag
5941  ttcttccagg acgtctgcaa gcccaagtac agcggcaccc tgaacctgga cagggtgacc
6001  cgagaggcgt gccctgagct ggactacttt gtggtcttct cctctgtgag ctgcgggcgt
6061  ggcaatgcgg gacagagcaa ctacggcttt gccaattccg ccatggagcg tatctgtgag
6121  aaacgccggc acgaaggcct cccaggcctg gccgtgcagt ggggcgccat cggcaccgtg
6181  ggcattttgg tggagacgat gagcaccaac gacacgatcg tcagtggcac gctgcccacg
6241  cgcattggcg tccttggcct ggaggtgctg gacctcttcc tgaaccagcc ccacatggtc
6301  ctgagcagct ttgtgctggc tgagaaggct gcggcctata gggacaggga cagccagcgg
6361  gacctggtgg aggccgtggc acacatcctg ggcatccgcg acttggctgc tgtcaacctg
6421  ggcggctcac tggcggacct gggcctggac tcgctcatga gcgccgccgt gcgccagacg
6481  ctggagcgtg agctcaacct ggtgctgtcc gtgcgcgagg tgcggcaact cacgctccgg
6541  aaactgcagg agctgtcctc aaaggcggat gaagccagcg agctggcatg ccccacgccc
6601  aaggaggatg gtctggccca gcagcagact cagctgaacc tgcgctccct gctggtgaaa
6661  ccggagggcc ccaccctgat gcggctcaac tccgtgcaga gctcggagcg gcccctgttc
6721  ctggtgcacc caatcgaggc taccaccgtg ttccacagcc tcggtcccgg tctcagcatc
6781  cccacctatg gcctgcagtg caccccggct gcgccccttg acagcatcca cagcctggct
6841  gcctactaca tcgactgcat caggcaggtg cagcccgagg gcccctaccg cgtggccggc
6901  tactcctacg gggcctgcgt ggcctttgaa atgtgctccc agctgcaggc ccagcagagc
6961  ccagccccca cccacaacag cctcttcctg ttcgacggct cgcccaccta cgtactggcc
7021  tacacccaga gctaccgggc aaagctgacc ccaggctgta aggctgaggc tgagacggag
7081  gccatatgct tcttcgtgca gcagttcacg gacatggagc acaacagggt gctggaggcg
7141  ctgctgccgc tgaagggcct agaggagcgt gtggcagccg ccgtggacct gatcatcaag
7201  agccaccagg gcctggaccg ccaggagctg agctttgcgg cccggtcctt ctactacagg
7261  ctgcgtgccg ctgaccagta tacacccaag gccaagtaca gtggcaacgt gatgctactg
7321  cgggccaaga cgggtggccg ctacggcgag gacctgggcg cggactacaa cctctcccag
7381  gtatgcgacg ggaaagtatc cgtccatatc atcgagggtg accaccgcac gctgctggag
7441  ggcagcggcc tggagtccat catcagcatc atccacagct ccctggctga gccacgtgtg
7501  agtcgggagg gctag
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SEQ ID NO:5

```
1     ctcaaaaggg gccggatttc cttctcctgg aggcagatgt tgcctctctc tctcgctcgg
61    attggttcag tgcactctag aaacactgct gtggtggaga aactggaccc caggtctgga
121   gcgaattcca gcctgcaggg ctgataagcg aggcattagt gagattgaga gagactttac
181   cccgccgtgg tggttggagg gcgcgcagta gagcagcagc acaggcgcgg gtcccgggag
241   gccggctctg ctcgcgccga gatgtggaat ctccttcacg aaaccgactc ggctgtggcc
301   accgcgcgcc gcccgcgctg gctgtgcgct ggggcgctgg tgctggcggg tggcttcttt
361   ctcctcggct tcctcttcgg gtggtttata aaatcctcca atgaagctac taacattact
421   ccaaagcata atatgaaagc atttttggat gaattgaaag ctgagaacat caagaagttc
481   ttatataatt ttacacagat accacattta gcaggaacag aacaaaactt tcagcttgca
541   aagcaaattc aatcccagtg gaaagaattt ggcctggatt ctgttgagct agcacattat
601   gatgtcctgt tgtcctaccc aaataagact catcccaact acatctcaat aattaatgaa
661   gatggaaatg agattttcaa cacatcatta tttgaaccac ctcctccagg atatgaaaat
721   gtttcggata ttgtaccacc tttcagtgct ttctctcctc aaggaatgcc agagggcgat
781   ctagtgtatg ttaactatgc acgaactgaa gacttcttta aattggaacg ggacatgaaa
841   atcaattgct ctgggaaaat tgtaattgcc agatatggga aagttttcag aggaaataag
901   gttaaaaatg cccagctggc aggggccaaa ggagtcattc tctactccga ccctgctgac
```

Figure 10 (cont.)

```
961   tactttgctc ctggggtgaa gtcctatcca gatggttgga atcttcctgg aggtggtgtc
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1081  gcaaatgaat atgcttatag gcgtggaatt gcagaggctg ttggtcttcc aagtattcct
1141  gttcatccaa ttggatacta tgatgcacag aagctcctag aaaaaatggg tggctcagca
1201  ccaccagata gcagctggag aggaagtctc aaagtgccct acaatgttgg acctggcttt
1261  actggaaact tttctacaca aaaagtcaag atgcacatcc actctaccaa tgaagtgaca
1321  agaatttaca atgtgatagg tactctcaga ggagcagtgg aaccagacag atatgtcatt
1381  ctgggaggtc accgggactc atgggtgttt ggtggtattg accctcagag tggagcagct
1441  gttgttcatg aaattgtgag gagctttgga acactgaaaa aggaagggtg gagacctaga
1501  agaacaattt tgtttgcaag ctgggatgca gaagaatttg gtcttcttgg ttctactgag
1561  tgggcagagg agaattcaag actccttcaa gagcgtggcg tggcttatat taatgctgac
1621  tcatctatag aaggaaacta cactctgaga gttgattgta caccgctgat gtacagcttg
1681  gtacacaacc taacaaaaga gctgaaaagc cctgatgaag gctttgaagg caaatctctt
1741  tatgaaagtt ggactaaaaa aagtccttcc ccagagttca gtggcatgcc caggataagc
1801  aaattgggat ctggaaatga ttttgaggtg ttcttccaac gacttggaat tgcttcaggc
1861  agagcacggt atactaaaaa ttgggaaaca aacaaattca gcggctatcc actgtatcac
1921  agtgtctatg aaacatatga gttggtggaa aagttttatg atccaatgtt taaatatcac
1981  ctcactgtgg cccaggttcg aggagggatg gtgtttgagc tagccaattc catagtgctc
2041  ccttttgatt gtcgagatta tgctgtagtt ttaagaaagt atgctgacaa aatctacagt
2101  atttctatga aacatccaca ggaaatgaag acatacagtg tatcatttga ttcacttttt
2161  tctgcagtaa agaattttac agaaattgct tccaagttca gtgagagact ccaggacttt
2221  gacaaaagca acccaatagt attaagaatg atgaatgatc aactcatgtt tctggaaaga
2281  gcatttattg atccattagg gttaccagac aggccttttt ataggcatgt catctatgct
2341  ccaagcagcc acaacaagta tgcaggggag tcattcccag gaatttatga tgctctgttt
2401  gatattgaaa gcaaagtgga cccttccaag gcctggggag aagtgaagag acagatttat
2461  gttgcagcct tcacagtgca ggcagctgca gagactttga gtgaagtagc ctaagaggat
2521  tctttagaga atccgtattg aatttgtgtg gtatgtcact cagaaagaat cgtaatgggt
2581  atattgataa attttaaaat tggtatattt gaaataaagt tgaatattat atataaaaaa
2641  aaaaaaaaaa aaa
```

SEQ ID NO:6

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121 ctgggacaga cctgcgtgct gatcgtgatc ttcacagtgc tcctgcagtc tctctgtgtg
181 gctgtaactt acgtgtactt taccaacgag ctgaagcaga tgcaggacaa gtactccaaa
241 agtggcattg cttgtttctt aaaagaagat gacagttatt gggaccccaa tgacgaagag
301 agtatgaaca gcccctgctg gcaagtcaag tggcaactcc gtcagctcgt tagaaagatg
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421 cccctagtga gagaaagagg tcctcagaga gtagcagctc acataactgg gaccagagga
481 agaagcaaca cattgtcttc tccaaactcc aagaatgaaa aggctctggg ccgcaaaata
541 aactcctggg aatcatcaag gagtgggcat tcattcctga gcaacttgca cttgaggaat
601 ggtgaactgg tcatccatga aaaagggttt tactacatct attcccaaac atactttcga
661 tttcaggagg aaataaaaga aaacacaaag aacgacaaac aaatggtcca atatatttac
721 aaatacacaa gttatcctga ccctatattg ttgatgaaaa gtgctagaaa tagttgttgg
781 tctaaagatg cagaatatgg actctattcc atctatcaag ggggaatatt tgagcttaag
841 gaaaatgaca gaatttttgt ttctgtaaca aatgagcact tgatagacat ggaccatgaa
901 gccagttttt ttggggcctt tttagttggc taactgacct ggaaagaaaa agcaataacc
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1321 agtagtagcc tccaggtttc cttaagggac aacatcctta agtcaaaaga gagaagaggc
```

Figure 10 (cont.)

```
1381 accactaaaa gatcgcagtt tgcctggtgc agtggctcac acctgtaatc ccaacatttt
1441 gggaacccaa ggtgggtaga tcacgagatc aagagatcaa gaccatagtg accaacatag
1501 tgaaacccca tctctactga aagtacaaaa attagctggg tgtgttggca catgcctgta
1561 gtcccagcta cttgagaggc tgaggcaaga gaattgtttg aacccgggag gcagaggttg
1621 cagtgtggtg agatcatgcc actacactcc agcctggcga cagagcgaga cttggtttca
1681 aaaaaaaaaa aaaaaaaaac ttcagtaagt acgtgttatt tttttcaata aaattctatt
1741 acagtatgtc

SEQ ID NO:7
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  61 gcgcggccgt tgccgaggag acggcgcatg tcccgccgcg cgttgccccc tctgcagtac
 121 ccccgccccc cttctcccac cacaatgaga tcctaagatg gcggtggctg cggcggttgg
 181 cgctgcgtag ctgaggtcga aaaggcggcc actggggccg aggcagccag gaaacgtgtg
 241 ggcctctctg ctgcggtctc cgagggccga ccgctgccgg cggcgggtcg tgggggctga
 301 ctgtcgctct gcctttgaca ggagaggctg cttcttgtag aggaaacagc tttgaagtgt
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 421 gttaaatcca gtatggccac aggaggaggt ccctttgaag atggcatgaa tgatcaggat
 481 ttaccaaact ggagtaatga gaatgttgat gacaggctca acaatatgga ttggggtgcc
 541 caacagaaga aagcaaatag atcatcagaa aagaataaga aaaagtttgg tgtagaaagt
 601 gataaaagag taaccaatga tatttctccg gagtcgtcac caggagttgg aaggcgaaga
 661 acaaagactc cacatacgtt cccacacagt agatacatga gtcagatgtc tgtcccagag
 721 caggcagaat tagagaaact gaaacagcgg ataaacttca gtgatttaga tcagagaagc
 781 attggaagtg attcccaagg tagagcaaca gctgctaaca acaaacgtca gcttagtgaa
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 901 tctacaagtc ccccaaacag agaaacgatt ggatcagcac agtgtaaaga gttgtttgct
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1021 gaacctgcaa tggagagcag ccagattgta agcaggcttg ttcaaattcg cgattatatt
1081 actaaagcta gttccatgcg ggaagatctt gtagagaaaa atgagagatc tgctaatgtt
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1261 aagaaacaac atgatttatt aaaaagaatg ttacaacagc aggagcaact aagagctcta
1321 cagggacggc aggctgcact tctagctctg caacataaag cagagcaagc tattgcagtg
1381 atggatgatt ctgttgttgc agaaactgca ggtagcttat ctggcgtcag tatcacatct
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1561 gtttcccaga gcaggaaacc atcagcttca gaacgtttac ctgatgagaa agtcgaactt
1621 tttagcaaaa tgagagtgct acaggaaaag aaacaaaaaa tggacaaatt gcttggagaa
1681 cttcatacac ttcgagatca gcatcttaac aattcatcat cctctccaca aaggagtgtc
1741 gatcagagaa gtacttcagc tcccctctgct tctgtaggct tggcaccggt tgtcaatgga
1801 gaatccaata gcctcacatc atctgttcct tatcctactg cttctctagt atctcagaat
1861 gagagtgaaa acgaaggcca cctcaatcca tctgaaaaac tccagaagtt aaatgaagtt
1921 cgaaagagat tgaatgagct aagagaatta gttcattatt atgaacaaac gtcagacatg
1981 atgacagatg ctgtgaatga aaacaggaaa gatgaagaaa ctgaagagtc agaatatgat
2041 tctgagcatg aaaattccga gcctgttact aacattcgaa atccacaagt agcttccact
2101 tggaatgaag taaatagtca tagtaatgca cagtgtgttt ctaataatag agatgggcga
2161 acagttaatt ctaattgtga aattaacaac agatctgctg ccaacataag ggctctaaac
2221 gtgcctcctt ctttagattg tcgatataat agagaagggg aacaggagat tcatgttgca
2281 caaggtgaag atgatgagga ggaggaggaa gaagcagaag aggagggagt cagtggagct
2341 tcattatcta gtcacaggag cagtctggtt gatgagcatc cagaagatgc tgaatttgaa
2401 cagaagatca accgacttat ggctgcaaaa cagaaactta gacagttaca agatcttgtt
2461 gctatggtac aggatgatga tgcagctcaa ggagttatct ctgccagtgc atcaaatttg
2521 gatgatttct acccagcaga agaagacacc aagcaaaatt caaataacac tagaggaaat
2581 gccaataaaa cacagaaaga tactggagta aatgaaaagg caagagagaa attttatgag
2641 gctaaactac agcagcaaca gagagagcta aaacaattgc aggaagaaag aaagaaactg
```

Figure 10 (cont.)

```
2701 attgacattc aggagaaaat tcaagcattg caaacggcat gccctgactt acagctgtca
2761 gctgctagtg tgggtaactg tcccaccaaa aaatatatgc cagctgttac ttcaacccca
2821 actgttaatc aacacgagac cagtacaagc aaatctgttt ttgagcctga agattcttca
2881 atagtagata atgagttgtg gtcagaaatg agaagacatg aaatgttgag ggaggagctg
2941 cgacagagaa gaaagcagct tgaagctctg atggctgaac atcagaggag gcaaggtcta
3001 gctgaaactg catctccagt ggctgtgtca ttgagaagtg atggatctga gaacctatgt
3061 actcctcagc aaagtagaac agaaaaaacg atggcaactt ggggagggtc tacccagtgt
3121 gcactagatg aagaaggaga tgaagacggt taccttttctg aaggaattgt tcggacagat
3181 gaagaggagg aagaagagca agatgccagt tccaatgata acttttctgt gtgtccttct
3241 racagtgtga atcataactc ctacaatgga aaggaaacta aaaataggtg gaagaacaat
3301 tgccctttt cggcagatga aaattatcgt cctttagcca agacaaggca acagaatatc
3361 agcatgcaac ggcaagaaaa ccttcgttgg gtgtcagagc tctcttacgt agaagagaaa
3421 gaacaatggc aagaacaaat caatcagcta aagaaacagc ttgattttag tgtcagtatt
3481 tgtcagactt tgatgcaaga ccagcagact ctatcttgtc tgctacaaac tcttctcacg
3541 ggtccttaca gtgttatgcc cagcaatgtt gcatctcctc aagtacactt cataatgcac
3601 cagttgaacc agtgctatac tcagctaaca tggcaacaga ataatgttca gaggttgaaa
3661 caaatgctaa atgaacttat gcgccagcaa aatcagcatc cagaaaaacc tggaggcaag
3721 gaaagaggca gtagtgcatc gcaccctcct tctcccagtt tattttgtcc tttcagcttt
3781 ccaacacagc ctgtaaatct cttcaatata cctggattta ctaacttttc atcatttgca
3841 ccaggtatga atttcagccc tttatttcct tctaattttg gagatttttc tcagaatatc
3901 tctacaccca gtgaacagca gcaaccctta gcccagaatt cttcaggaaa aacagaatat
3961 atggcttttc caaaaccttt tgaaagcagt tcctctattg gagcagagaa accaaggaat
4021 aaaaaactgc ctgaagagga ggtggaaagc agtaggacac catggttata tgaacaagaa
4081 ggtgaagtag agaaaccatt tatcaagact ggattttcag tgtctgtaga aaaatctaca
4141 agtagtaacc gcaaaaatca attagataca aacggaagaa gacgccagtt tgatgaagaa
4201 tcactggaaa gctttagcag tatgcctgat ccagtagatc caacaacagt gactaaaaca
4261 ttcaagacaa gaaaagcgtc tgcacaggcc agcctggcat ctaaagataa aactcccaag
4321 tcaaaaagta agaagaggaa ttctactcag ctgaaaagca gagttaaaaa catcaggtat
4381 gaaagtgcca gtatgtctag cacatgtgaa ccttgcaaaa gtaggaacag acattcagcc
4441 cagactgaag agcctgttca agcaaaagta ttcagcagaa agaatcatga gcaactggaa
4501 aaaataataa aatgtaatag gtctacagaa atatcttcag aaactgggag tgatttttcc
4561 atgtttgaag ctttgcgaga tactatttat tctgaagtag ctacattaat ttctcaaaat
4621 gaatctcgtc cacattttct tattgaactc ttccatgagc tgcagctact aaacacagac
4681 tacttgagac agagggcttt atatgcattg caggacatag tatccagaca tatttctgag
4741 agccatgaaa aaggagaaaa tgtaaagtca gtaaactctg gtacttggat agcatcaaac
4801 tcagaactta ctcctagtga gagccttgct actactgatg atgaaacttt tgagaagaac
4861 tttgaaagag aaacccataa aataagtgag caaaatgatg ctgataatgc tagtgtcctg
4921 tctgtatcat caaattttga gcctttgca acagatgatc taggtaacac cgtgattcac
4981 ttagatcaag cattagccag aatgagagaa tatgagcgta tgaagactga ggctgaaagt
5041 aactcaaata tgagatgcat ctgcaggatt attgaggatg gagatggtgc tggtgcaggt
5101 actacagtta ataatttaga agaaactccc gttattgaaa atcgtagttc acaacaacct
5161 gtaagtgaag tttctaccat cccatgtcct agaattgata ctcagcagct ggaccggcaa
5221 attaaagcaa ttatgaaaga agtcattcct tttttgaagg agcacatgga tgaagtatgc
5281 tcctcgcagc ttctaacttc agtaaggcgc atggttttga cccttaccca gcaaaatgat
5341 gagagcaaag agtttgtaaa gttctttcat aaacaacttg gaagtatatt acaggattca
5401 ctggcaaaat ttgctggcag aaaactgaaa gactgtggag aagatcttct tgtagagata
5461 tctgaagtgt tgttcaatga attggctttc tttaagctta tgcaagattt ggataataat
5521 agtataactg ttaaacagag atgcaaaagg aaaatagaag caactggagt gatacaatct
5581 tgtgccaaag agctaaaagg attcttgaag atcatggctc acctgctgga gagattgatg
5641 atgaagacaa agacaaggat gaaactgaaa cagttaagca gactcaaaca tctgaggtgt
5701 atgatggtcc caaaaatgta agatctgata tttctgatca agaggaagat gaagaaagtg
5761 aaggatgtcc agtgtctatt aatttgtcta aagctgaaac tcaggcttta actaattatg
5821 gaagtggaga agatgaaaat gaggatgaag aaatggaaga atttgaagaa ggccctgtgg
5881 atgtccagac ttccctccag gctaacactg aagctactga agaaaatgaa catgatgaac
```

Figure 10 (cont.)

```
5941 aggtcctaca acgtgacttt aaaaagacag cagaaagcaa aaatgtccca ttggaacgag
6001 aagccactag taaaaatgac caaaataact gtcctgtgaa accctgttac ctcaatatct
6061 tggaagatga gcaaccttta aatagtgctg cccataagga gtcacctcct actgttgatt
6121 caactcaaca gcctaaccct ttgccgttac gtttacctga aatggaaccc ttagtgccta
6181 gagtcaaaga agttaaatct gctcaggaaa ctcctgaaag ctctctggct ggaagtcctg
6241 atactgaatc tccagtgtta gtgaatgact atgaagcaga atctggtaat ataagtcaaa
6301 agtctgatga agaagatttt gtaaaagttg aagatttacc actgaaactg acaatatatt
6361 cagaggcaga tctaagaaag aaaatggtag aagaagaaca gaaaaaccat ttatctggtg
6421 aaatatgtga aatgcagacc gaagaattag ctggaaattc tgagacacta aaagaacctg
6481 aaacggtggg agcccagagt atatgagatg tcttcagagg ctcatctaac tctgtcctta
6541 catactcaat gcatatatga aaacaatact aaataaacat ctgatctgta taaaaat
```

SEQ ID NO:8

```
   1 ctccaaaggc aaaaatctcc agccctacag agactgagcg gtgcatcgag tccctgattg
  61 ctgtcttcca gaagtatgct ggaaaggatg gttataacta cactctctcc aagacagagt
 121 tcgtaagctt catgaataca gaactagctg ccttcacaaa gaaccagaag gaccctggtg
 181 tccttgaccg catgatgaag aaactggaca ccaacagtga tggtcagcta gatttctcag
 241 aatttcttaa tctgattggt ggcctagcta tggcttgcca tgactccttc ctcaaggctg
 301 tcccttccca gaagcggacc tgaggacccc ttggccctgg ccttcaaacc cacccccttt
 361 ccttccagcc tttctgtcat catctccaca gcccacccat cccctgagca cactaaccac
 421 ctcatgcagg ccccacctgc caatagtaat aaagcaatgt cacttttta aaacatgaa
```

SEQ ID NO:9

```
1 gccgcttcct gcctggattc cacagcttcg cgccgtgtac tgtcgcccca tccctgcgcg
61 cccagcctgc caagcagcgt gccccggttg caggcgtcat gcagcgggcg cgacccacgc
121 tctgggccgc tgcgctgact ctgctggtgc tgctccgcgg gccgccggtg gcgcgggctg
181 gcgcgagctc ggcgggcttg ggtcccgtgg tgcgctgcga gccgtgcgac gcgcgtgcac
241 tggcccagtg cgcgcctccg cccgccgtgt gcgcggagct ggtgcgcgag ccgggctgcg
301 gctgctgcct gacgtgcgca ctgagcgagg gccagccgtg cggcatctac accgagcgct
361 gtggctccgg ccttcgctgc cagccgtcgc ccgacgaggc gcgaccgctg caggcgctgc
421 tggacggccg cgggctctgc gtcaacgcta gtgccgtcag ccgcctgcgc gcctacctgc
481 tgccagcgcc gccagctcca ggaaatgcta gtgagtcgga ggaagaccgc agcgccggca
541 gtgtggagag cccgtccgtc tccagcacgc accgggtgtc tgatcccaag ttccaccccc
601 tccattcaaa gataatcatc atcaagaaag ggcatgctaa agacagccag cgctacaaag
661 ttgactacga gtctcagagc acagataccc agaacttctc ctccgagtcc aagcgggaga
721 cagaatatgg tccctgccgt agagaaatgg aagacacact gaatcacctg aagttcctca
781 atgtgctgag tcccaggggt gtacacattc ccaactgtga caagaaggga ttttataaga
841 aaaagcagtg tcgcccttcc aaaggcagga agcggggctt ctgctggtgt gtggataagt
901 atgggcagcc tctcccaggc tacaccacca aggggaagga ggacgtgcac tgctacagca
961 tgcagagcaa gtagacgcct gccgcaaggt taatgtggag ctcaaatatg ccttattttg
1021 cacaaaagac tgccaaggac atgaccagca gctggctaca gcctcgattt atatttctgt
1081 ttgtggtgaa ctgatttttt ttaaaccaaa gtttagaaag aggtttttga aatgcctatg
1141 gtttctttga atggtaaact tgagcatctt ttcactttcc agtagtcagc aaagagcagt
1201 ttgaattttc ttgtcgcttc ctatcaaaat attcagagac tcgagcacag cacccagact
1261 tcatgcgccc gtggaatgct caccacatgt tggtcgaagc ggccgaccac tgactttgtg
1321 acttaggcgg ctgtgttgcc tatgtagaga acacgcttca cccccactcc ccgtacagtg
1381 cgcacaggct ttatcgagaa taggaaaacc tttaaacccc ggtcatccgg acatcccaac
1441 gcatgctcct ggagctcaca gccttctgtg gtgtcatttc tgaaacaagg gcgtggatcc
1501 ctcaaccaag aagaatgttt atgtcttcaa gtgacctgta ctgcttgggg actattggag
1561 aaaataaggt ggagtcctac ttgtttaaaa aatatgtatc taagaatgtt ctagggcact
1621 ctgggaacct ataaaggcag gtatttcggg ccctcctctt caggaatctt cctgaagaca
1681 tggcccagtc gaaggcccag gatggctttt gctgcggccc cgtggggtag gagggacaga
1741 gagacaggga gagtcagcct ccacattcag aggcatcaca agtaatggca caattcttcg
1801 gatgactgca gaaaatagtg ttttgtagtt caacaactca agacgaagct tatttctgag
```

Figure 10 (cont.)

```
1861 gataagctct ttaaaggcaa agctttattt tcatctctca tcttttgtcc tccttagcac
1921 aatgtaaaaa agaatagtaa tatcagaaca ggaaggagga atggcttgct ggggagccca
1981 tccaggacac tgggagcaca tagagattca cccatgtttg ttgaacttag agtcattctc
2041 atgcttttct ttataattca cacatatatg cagagaagat atgttcttgt taacattgta
2101 tacaacatag ccccaaatat agtaagatct atactagata atcctagatg aaatgttaga
2161 gatgctattt gatacaactg tggccatgac tgaggaaagg agctcacgcc cagagactgg
2221 gctgctctcc cggaggccaa acccaagaag gtctggcaaa gtcaggctca gggagactct
2281 gccctgctgc agacctcggt gtggacacac gctgcataga gctctccttg aaaacagagg
2341 ggtctcaaga cattctgcct acctattagc ttttctttat ttttttaact ttttgggggg
2401 aaaagtattt ttgagaagtt tgtcttgcaa tgtatttata aatagtaaat aaagttttta
2461 ccatt
```

SEQ ID NO:10

```
   1 atgccgcgct ccttcctggt caagaagcat ttcaacgcct ccaaaaagcc aaactacagc
  61 gaactggaca cacatacagt gattatttcc ccgtatctct atgagagtta ctccatgcct
 121 gtcataccac aaccagagat cctcagctca ggagcataca gccccatcac tgtgtggact
 181 accgctgctc cattccacgc ccagctaccc aatggcctct ctcctctttc cggatactcc
 241 tcatctttgg ggcgagtgag tccccctcct ccatctgaca cctcctccaa ggaccacagt
 301 ggctcagaaa gccccattag tgatgaagag gaaagactac agtccaagct ttcagacccc
 361 catgccattg aagctgaaaa gtttcagtgc aatttatgca ataagaccta ttcaactttt
 421 tctgggctgg ccaaacataa gcagctgcac tgcgatgccc agtctagaaa atctttcagc
 481 tgtaaatact gtgacaagga atatgtgagc ctgggcgccc tgaagatgca tattcggacc
 541 cacacattac cttgtgtttg caagatctgc ggcaaggcgt tttccagacc ctggttgctt
 601 caaggacaca ttagaactca cacgggggag aagccttttt cttgccctca ctgcaacaga
 661 gcatttgcag acaggtcaaa tctgagggct catctgcaga cccattctga tgtaaagaaa
 721 taccagtgca aaaactgctc caaaaccttc tccagaatgt ctctcctgca caaacatgag
 781 gaatctggct gctgtgtagc acactga
```

SEQ ID NO:11

```
   1 ctcggaagcc cgtcaccatg tcgtgcgagt cgtctatggt tctcgggtac tgggatattc
  61 gtgggctggc gcacgccatc cgcctgctcc tggagttcac ggatacctct tatgaggaga
 121 aacggtacac gtgcggggaa gctcctgact atgatcgaag ccaatggctg gatgtgaaat
 181 tcaagctaga cctggacttt cctaatctgc cctacctcct ggatgggaag aacaagatca
 241 cccagagcaa tgccatcttg cgctacatcg ctcgcaagca caacatgtgt ggtgagactg
 301 aagaagaaaa gattcgagtg gacatcatag agaaccaagt aatggatttc cgcacacaac
 361 tgataaggct ctgttacagc tctgaccacg aaaaactgaa gcctcagtac ttggaagagc
 421 tacctggaca actgaaacaa ttctccatgt ttctgtggaa attctcatgg tttgccgggg
 481 aaaagctcac ctttgtggat tttctcacct atgatatctt ggatcagaac cgtatatttg
 541 accccaagtg cctggatgag ttcccaaacc tgaaggcttt catgtgccgt tttgaggctt
 601 tggagaaaat cgctgcctac ttacagtctg atcagttctg caagatgccc atcaacaaca
 661 agatggccca gtggggcaac aagcctgtat gctgagcagg aggcagactt gcagagcttg
 721 ttttgtttca tcctgtccgt aaggggtcag cgctcttgct ttgctctttt caatgaatag
 781 cacttatgtt actggtgtcc agctgagttt ctcttgggta taaaggctaa aagggaaaaa
 841 ggatatgtgg agaatcatca agatatgaat tgaatcgctg cgatactgtg gcatttccct
 901 actccccaac tgagttcaag ggctgtaggt tcatgcccaa gccctgagag tgggtactag
 961 aaaaaacgag attgcacagt tggagagagc aggtgtgtta aatggactgg agtccctgtg
1021 aagactgggt gaggataaca caagtaaaac tgtggtactg atggacttaa ccggagttcg
1081 gaaaccgtcc tgtgtacaca tgggagttta gtgtgataaa ggcagtattt cagactggtg
1141 ggctagccaa tagagttggc aattgcttat tgaaactcat taaaaataat agagccccac
1201 ttgacactat tcactaaaat taatctggaa tttaaggccc aacattaaac acaaagctgt
1261 attgat
```

SEQ ID NO:12

```
   1 gccacgtgct gctgggtctc agtcctccac ttcccgtgtc ctctggaagt tgtcaggagc
```

Figure 10 (cont.)

```
  61 aatgttgcgc ttgtacgtgt tggtaatggg agtttctgcc ttcacccttc agcctgcggc
 121 acacacaggg gctgccagaa gctgccggtt tcgtgggagg cattacaagc gggagttcag
 181 gctggaaggg gagcctgtag ccctgaggtg cccccaggtg ccctactggt tgtgggcctc
 241 tgtcagcccc cgcatcaacc tgacatggca taaaaatgac tctgctagga cggtcccagg
 301 agaagaagag acacggatgt gggcccagga cggtgctctg tggcttctgc cagccttgca
 361 ggaggactct ggcacctacg tctgcactac tagaaatgct tcttactgtg acaaaatgtc
 421 cattgagctc agagtttttg agaatacaga tgctttcctg ccgttcatct catacccgca
 481 aattttaacc ttgtcaacct ctggggtatt agtatgccct gacctgagtg aattcacccg
 541 tgacaaaact gacgtgaaga ttcaatggta caaggattct cttcttttgg ataaagacaa
 601 tgagaaattt ctaagtgtga gggggaccac tcacttactc gtacacgatg tggccctgga
 661 agatgctggc tattaccgct gtgtcctgac atttgcccat gaaggccagc aatacaacat
 721 cactaggagt attgagctac gcatcaagaa aaaaaaagaa gagaccattc ctgtgatcat
 781 ttcccccctc aagaccatat cagcttctct ggggtcaaga ctgacaatcc cgtgtaaggt
 841 gtttctggga accggcacac ccttaaccac catgctgtgg tggacggcca atgacaccca
 901 catagagagc gcctacccgg gaggccgcgt gaccgagggg ccacgccagg aatattcaga
 961 aaataatgag aactacattg aagtgccatt gatttttgat cctgtcacaa gagaggattt
1021 gcacatggat tttaaatgtg ttgtccataa taccctgagt tttcagacac tacgcaccac
1081 agtcaaggaa gcctcctcca cgttctcctg gggcattgtg ctggccccac tttcactggc
1141 cttcttggtt ttgggggaa tatggatgca cagacggtgc aaacacagaa ctggaaaagc
1201 agatggtctg actgtgctat ggcctcatca tcaagacttt caatcctatc ccaagtgaaa
1261 taaatggaat gaaataattc aaacacaaaa aaaaaaaaaa aaaaaaaa
```

SEQ ID NO:13

```
1 gcgctgcccg cctcgtcccc acccccaac ccccgcgcc cgccctcgga cagtccctgc
61 tcgcccgcgc gctgcagccc catctcctag cggcagccca ggcgcggagg gagcgagtcc
121 gccccgaggt aggtccagga cgggcgcaca gcagcagccg aggctggccg ggagagggag
181 gaagaggatg gcagggccac gccccagccc atgggccagg ctgctcctgg cagccttgat
241 cagcgtcagc ctctctggga ccttggcaaa ccgctgcaag aaggccccag tgaagagctg
301 cacggagtgt gtccgtgtgg ataaggactg cgcctactgc acagacgaga tgttcaggga
361 ccggcgctgc aacacccagg cggagctgct ggccgcgggc tgccagcggg agagcatcgt
421 ggtcatggag agcagcttcc aaatcacaga ggagacccag attgacacca ccctgcggcg
481 cagccagatg tccccccaag gcctgcgggt ccgtctgcgg cccggtgagg agcggcattt
541 tgagctggag gtgtttgagc cactggagag ccccgtggac ctgtacatcc tcatggactt
601 ctccaactcc atgtccgatg atctggacaa cctcaagaag atggggcaga acctggctcg
661 ggtcctgagc cagctcacca gcgactacac tattggattt ggcaagtttg tggacaaagt
721 cagcgtcccg cagacggaca tgaggcctga gaagctgaag gagccctggc ccaacagtga
781 ccccccttc tccttcaaga acgtcatcag cctgacagaa gatgtggatg agttccggaa
841 taaactgcag ggagagcgga tctcaggcaa cctggatgct cctgagggcg gcttcgatgc
901 catcctgcag acagctgtgt gcacgaggga cattggctgg cgcccggaca gcacccacct
961 gctggtcttc tccaccgagt cagccttcca ctatgaggct gatggcgcca acgtgctggc
1021 tggcatcatg agccgcaacg atgaacggtg ccacctggac accacgggca cctacaccca
1081 gtacaggaca caggactacc cgtcggtgcc caccctggtg cgcctgctcg ccaagcacaa
1141 catcatcccc atctttgctg tcaccaacta ctcctatagc tactacgaga agcttcacac
1201 ctatttccct gtctcctcac tgggggtgct gcaggaggac tcgtccaaca tcgtggagct
1261 gctggaggag gccttcaatc ggatccgctc caacctggac atccgggccc tagacagccc
1321 ccgaggcctt cggacagagg tcacctccaa gatgttccag aagacgagga ctgggtcctt
1381 tcacatccgg cgggggaag tgggtatata ccaggtgcag ctgcgggccc ttgagcacgt
1441 ggatgggacg cacgtgtgcc agctgccgga ggaccagaag ggcaacatcc atctgaaacc
1501 ttccttctcc gacggcctca agatggacgc gggcatcatc tgtgatgtgt gcacctgcga
1561 gctgcaaaaa gaggtgcggt cagctcgctg cagcttcaac ggagacttcg tgtgcggaca
1621 gtgtgtgtgc agcgagggct ggagtggcca gacctgcaac tgctccaccg gctctctgag
1681 tgacattcag ccctgcctgc gggagggcga ggacaagccg tgctccggcc gtggggagtg
1741 ccagtgcggg cactgtgtgt gctacggcga aggccgctac gagggtcagt tctgcgagta
1801 tgacaacttc cagtgtcccc gcacttccgg gttcctgtgc aatgaccgag gacgctgctc
```

Figure 10 (cont.)

```
1861 catgggccag tgtgtgtgtg agcctggttg gacaggccca agctgtgact gtcccctcag
1921 caatgccacc tgcatcgaca gcaatggggg catctgtaat ggacgtggcc actgtgagtg
1981 tggccgctgc cactgccacc agcagtcgct ctacacggac accatctgcg agatcaacta
2041 ctcggcgatc cacccgggcc tctgcgagga cctacgctcc tgcgtgcagt gccaggcgtg
2101 gggcaccggc gagaagaagg ggcgcacgtg tgaggaatgc aacttcaagg tcaagatggt
2161 ggacgagctt aagagagccg aggaggtggt ggtgcgctgc tccttccggg acgaggatga
2221 cgactgcacc tacagctaca ccatggaagg tgacggcgcc cctgggccca acagcactgt
2281 cctggtgcac aagaagaagg actgccctcc gggctccttc tggtggctca tccccctgct
2341 cctcctcctc ctgccgctcc tggccctgct actgctgcta tgctggaagt actgtgcctg
2401 ctgcaaggcc tgcctggcac ttctcccgtg ctgcaaccga ggtcacatgg tgggctttaa
2461 ggaagaccac tacatgctgc gggagaacct gatggcctct gaccacttgg acacgcccat
2521 gctgcgcagc gggaacctca agggccgtga cgtggtccgc tggaaggtca ccaacaacat
2581 gcagcggcct ggctttgcca ctcatgccgc cagcatcaac cccacagagc tggtgcccta
2641 cgggctgtcc ttgcgcctgg cccgcctttg caccgagaac ctgctgaagc ctgacactcg
2701 ggagtgcgcc cagctgcgcc aggaggtgga ggagaacctg aacgaggtct acaggcagat
2761 ctccggtgta cacaagctcc agcagaccaa gttccggcag cagcccaatg ccgggaaaaa
2821 gcaagaccac accattgtgg acacagtgct gatggcgccc cgctcggcca agccggccct
2881 gctgaagctt acagagaagc aggtggaaca gagggccttc cacgacctca aggtggcccc
2941 cggctactac accctcactg cagaccagga cgcccggggc atggtggagt tccaggaggg
3001 cgtggagctg gtggacgtac gggtgccccc ctttatccgg cctgaggatg acgacgagaa
3061 gcagctgctg gtggaggcca tcgacgtgcc cgcaggcact gccaccctcg gccgccgcct
3121 ggtaaacatc accatcatca aggagcaagc cagagacgtg gtgtcctttg agcagcctga
3181 gttctcggtc agccgcgggg accaggtggc ccgcatccct gtcatccggc gtgtcctgga
3241 cggcgggaag tcccaggtct cctaccgcac acaggatggc accgcgcagg gcaaccggga
3301 ctacatcccc gtggagggtg agctgctgtt ccagcctggg gaggcctgga aagagctgca
3361 ggtgaagctc ctggagctgc aagaagttga ctccctcctg cggggccgcc aggtccgccg
3421 tttccacgtc cagctcagca accctaagtt tggggcccac ctgggccagc cccactccac
3481 caccatcatc atcagggacc cagatgaact ggaccggagc ttcacgagtc agatgttgtc
3541 atcacagcca ccccctcacg gcgacctggg cgccccgcag aaccccaatg ctaaggccgc
3601 tgggtccagg aagatccatt tcaactggct gcccccttct ggcaagccaa tggggtacag
3661 ggtaaagtac tggattcagg gcgactccga atccgaagcc cacctgctcg acagcaaggt
3721 gccctcagtg gagctcacca acctgtaccc gtattgcgac tatgagatga aggtgtgcgc
3781 ctacgggggct cagggcgagg gaccctacag ctccctggtg tcctgccgca cccaccagga
3841 agtgcccagc gagccagggc gtctggcctt caatgtcgtc tcctccacgg tgacccagct
3901 gagctgggct gagccggctg agaccaacgg tgagatcaca gcctacgagg tctgctatgg
3961 cctggtcaac gatgacaacc gacctattgg gcccatgaag aaagtgctgg ttgacaaccc
4021 taagaaccgg atgctgctta ttgagaacct tcgggagtcc cagccctacc gctacacggt
4081 gaaggcgcgc aacggggccg gctgggggcc tgagcgggag gccatcatca acctggccac
4141 ccagcccaag aggcccatgt ccatccccat catccctgac atccctatcg tggacgccca
4201 gagcggggag gactacgaca gcttccttat gtacagcgat gacgttctac gctctccatc
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4321 gctgggggag gagctggacc tgcggcgcgt cacgtggcgg ctgcccccgg agctcatccc
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4441 gacggcggcg cgggcgggaa gggcggcagc cgtgccccgc agtgcgacac ccgggccccc
4501 cggagagcac ctggtgaatg gccggatgga ctttgccttc ccgggcagca ccaactccct
4561 gcacaggatg accacgacca gtgctgctgc ctatggcacc cacctgagcc cacacgtgcc
4621 ccaccgcgtg ctaagcacat cctccaccct cacacgggac tacaactcac tgacccgctc
4681 agaacactca cactcgacca cactgcccag ggactactcc accctcacct ccgtctcctc
4741 ccacgactct cgcctgactg ctggtgtgcc cgacacgccc acccgcctgg tgttctctgc
4801 cctggggccc acatctctca gagtgagctg gcaggagccg cggtgcgagc ggccgctgca
4861 gggctacagt gtggagtacc agctgctgaa cggcggtgag ctgcatcggc tcaacatccc
4921 caaccctgcc cagacctcgg tggtggtgga agacctcctg cccaaccact cctacgtgtt
4981 ccgcgtgcgg gcccagagcc aggaaggctg gggccgagag cgtgagggtg tcatcaccat
5041 tgaatcccag gtgcacccgc agagcccact gtgtcccctg ccaggctccg ccttcacttt
```

Figure 10 (cont.)

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5101 gagcactccc agtgccccag gcccgctggt gttcactgcc ctgagcccag actcgctgca
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5221 tgagatggcc caaggaggag ggccagccac cgcattccgg gtggatggag acagccccga
5281 gagccggctg accgtgccgg gcctcagcga gaacgtgccc tacaagttca aggtgcaggc
5341 caggaccact gagggcttcg ggccagagcg cgagggcatc atcaccatag agtcccagga
5401 tggaggaccc ttcccgcagc tgggcagccg tgccgggctc ttccagcacc cgctgcaaag
5461 cgagtacagc agcatcacca ccacccacac cagcgccacc gagcccttcc tagtggatgg
5521 gctgaccctg ggggcccagc acctggaggc aggcggctcc ctcacccggc atgtgaccca
5581 ggagtttgtg agccggacac tgaccaccag cggaaccctt agcacccaca tggaccaaca
5641 gttcttccaa acttgaccgc acccctgccc acccccgcca tgtcccacta ggcgtcctcc
5701 cgactcctct cccggagcct cctcagctac tccatccttg caccccctggg ggcccagccc
5761 acccgcatgc acagagcagg ggctaggtgt ctcctgggag gcatgaaggg ggcaaggtcc
5821 gtcctctgtg ggcccaaacc tatttgtaac caaagagctg ggagcagcac aaggacccag
5881 cctttgttct gcacttaata aatggttttg ctactgctaa aaaaaaaaaa aaaaaaaaaa
5941 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa
```

SEQ ID NO:14

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1 ccgccgggct ggccatggag ctgctgtgcc acgaggtgga cccggtccgc agggccgtgc
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121 gctaccttcc gcagtgctcc tacttcaagt gcgtgcagaa ggacatccaa ccctacatgc
181 gcagaatggt ggccacctgg atgctggagg tctgtgagga acagaagtgc gaagaagagg
241 tcttccctct ggccatgaat tacctggacc gtttcttggc tggggtcccg actccgaagt
301 cccatctgca actcctgggt gctgtctgca tgttcctggc ctccaaactc aaagagacca
361 gcccgctgac cgcggagaag ctgtgcattt acaccgacaa ctccatcaag cctcaggagc
421 tgctggagtg ggaactggtg gtgctgggga agttgaagtg gaacctggca gctgtcactc
481 ctcatgactt cattgagcac atcttgcgca agctgcccca gcagcgggag aagctgtctc
541 tgatccgcaa gcatgctcag accttcattg ctctgtgtgc caccgacttt aagtttgcca
601 tgtacccacc gtcgatgatc gcaactggaa gtgtgggagc agccatctgt gggctccagc
661 aggatgagga agtgagctcg ctcacttgtg atgccctgac tgagctgctg gctaagatca
721 ccaacacaga cgtggattgt ctcaaagctt gccaggagca gattgaggcg gtgctcctca
781 atagcctgca gcagtaccgt caggaccaac gtgacggatc caagtcggag gatgaactgg
841 accaagccag cacccctaca gacgtgcggg atatcgacct gtgaggatgc cagttgggcc
901 gaaagagaga gacgcgtcca taatctggtc tcttcttctt tctggttgtt tttgttcttt
961 gtgttttagg gtgaaactta aaaaaaaaat tctgccccca cctagatcat atttaaagat
1021 cttttagaag tgagagaaaa aggtcctacg aaaacggaat aataaaaagc atttggtgcc
1081 tatttgaagt acagcataag ggaatccctt gtatatgcga acagttattg tttgattatg
1141 taaaagtaat agtaaaatgc ttacaggaaa acctgcagag tagttagaga atatgtatgc
1201 ctgcaatatg ggaacaaatt agaggagact ttttttttc atgttatgag ctagcacata
1261 cacccccttg tagtataatt tcaaggaact gtgtacgcca tttatggcat gattagattg
1321 caaagcaatg aactcaagaa ggaattgaaa taaggaggga catgatgggg aaggagtaca
1381 aaacaatctc tcaacatgat tgaaccattt gggatggaga agcacctttg ctctcagcca
1441 cctgttacta agtcaggagt gtagttggat ctctacatta atgtcctctt gctgtctaca
1501 gtagctgcta cctaaaaaaa gatgttttat tttgccagtt ggacacaggt gattggctcc
1561 tgggtttcat gttctgtgac atcctgcttc ttcttccaaa tgcagttcat tgcagacacc
1621 accatattgc tatctaatgg ggaaatgtag ctatgggcca taaccaaaac tcacatgaaa
1681 cggaggcaga tggagaccaa gggtgggatc cagaatggag tcttttctgt tattgtattt
1741 aaaagggtaa tgtggccttg gcatttcttc ttagaaaaaa actaattttt ggtgctgatt
1801 ggcatgtctg gttcacagtt tagcattgtt ataaaccatt ccattcgaaa agcactttga
1861 aaaattgttc ccgagcgata gatgggatgg tttatgca
```

SEQ ID NO:15

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1 gagacattcc ggtgggggac tctggccagc ccgagcaacg tggatcctga gagcactccc
61 aggtaggcat ttgccccggt gggacgcctt gccagagcag tgtgtggcag gcccccgtgg
121 aggatcaaca cagtggctga acactgggaa ggaactggta cttggagtct ggacatctga
```

Figure 10 (cont.)

```
181 aacttggctc tgaaactgcg cagcggccac cggacgcctt ctggagcagg tagcagcatg
241 cagccgcctc caagtctgtg cggacgcgcc ctggttgcgc tggttcttgc ctgcggcctg
301 tcgcggatct ggggagagga gagaggcttc ccgcctgaca gggccactcc gcttttgcaa
361 accgcagaga taatgacgcc acccactaag accttatggc ccaagggttc caacgccagt
421 ctggcgcggt cgttggcacc tgcggaggtg cctaaaggag acaggacggc aggatctccg
481 ccacgcacca tctcccctcc cccgtgccaa ggacccatcg agatcaagga gactttcaaa
541 tacatcaaca cggttgtgtc ctgccttgtg ttcgtgctgg ggatcatcgg gaactccaca
601 cttctgagaa ttatctacaa gaacaagtgc atgcgaaacg gtcccaatat cttgatcgcc
661 agcttggctc tgggagacct gctgcacatc gtcattgaca tccctatcaa tgtctacaag
721 ctgctggcag aggactggcc atttggagct gagatgtgta agctggtgcc tttcatacag
781 aaagcctccg tgggaatcac tgtgctgagt ctatgtgctc tgagtattga cagatatcga
841 gctgttgctt cttggagtag aattaaagga attggggttc caaaatggac agcagtagaa
901 attgttttga tttgggtggt ctctgtggtt ctggctgtcc ctgaagccat aggttttgat
961 ataattacga tggactacaa aggaagttat ctgcgaatct gcttgcttca tcccgttcag
1021 aagacagctt tcatgcagtt ttacaagaca gcaaaagatt ggtggctgtt cagtttctat
1081 ttctgcttgc cattggccat cactgcattt ttttatacac taatgacctg tgaaatgttg
1141 agaaagaaaa gtggcatgca gattgcttta aatgatcacc taaagcagag acgggaagtg
1201 gccaaaaccg tcttttgcct ggtccttgtc tttgccctct gctggcttcc ccttcacctc
1261 agcaggattc tgaagctcac tctttataat cagaatgatc ccaatagatg tgaacttttg
1321 agctttctgt tggtattgga ctatattggt atcaacatgg cttcactgaa ttcctgcatt
1381 aacccaattg ctctgtattt ggtgagcaaa agattcaaaa actgctttaa gtcatgctta
1441 tgctgctggt gccagtcatt tgaagaaaaa cagtccttgg aggaaaagca gtcgtgctta
1501 aagttcaaag ctaatgatca cggatatgac aacttccgtt ccagtaataa atacagctca
1561 tcttgaaaga agaactattc actgtatttc attttcttta tattggaccg aagtcattaa
1621 aacaaaatga aacatttgcc aaaacaaaac aaaaaactat gtatttgcac agcacactat
1681 taaaatatta agtgtaatta ttttaacact cacagctaca tatgacattt tatgagctgt
1741 ttacggcatg gaaagaaaat cagtgggaat taagaaagcc tcgtcgtgaa agcacttaat
1801 tttttacagt tagcacttca acatagctct taacaacttc caggatattc acacaacact
1861 taggcttaaa aatgagctca ctcagaattt ctattctttc taaaaagaga tttatttta
1921 aatcaatggg actctgatat aaaggaagaa taagtcactg taaaacagaa cttttaaatg
1981 aagcttaaat tactcaattt aaaattttaa aatcctttaa aacaactttt caattaatat
2041 tatcacacta ttatcagatt gtaattagat gcaaatgaga gagcagttta gttgttgcat
2101 tttcggaca ctggaaacat ttaaatgatc aggagggagt aacagaaaga gcaaggctgt
2161 ttttgaaaat cattacactt tcactagaag cccaaacctc agcattctgc aatatgtaac
2221 caacatgtca caaacaagca gcatgtaaca gactggcaca tgtgccagct gaatttaaaa
2281 tataatactt ttaaaaagaa aattattaca tcctttacat tcagttaaga tcaaacctca
2341 caaagagaaa tagaatgttt gaaaggctat cccaaaagac ttttttgaat ctgtcattca
2401 cataccctgt gaagacaata ctatctacaa ttttttcagg attattaaaa tcttcttttt
2461 tcactatcgt agcttaaact ctgtttggtt ttgtcatctg taaatactta cctacataca
2521 ctgcatgtag atgattaaat gagggcaggc cctgtgctca tagctttacg atggagagat
2581 gccagtgacc tcataataaa gactgtgaac tgcctggtgc agtgtccaca tgacaaaggg
2641 gcaggtagca ccctctctca cccatgctgt ggttaaaatg gtttctagca tatgtataat
2701 gctatagtta aaatactatt tttcaaaatc atacagatta gtacatttaa cagctacctg
2761 taaagcttat tactaatttt tgtattattt ttgtaaatag ccaatagaaa agtttgcttg
2821 acatggtgct tttctttcat ctagaggcaa aactgctttt tgagaccgta agaacctctt
2881 agctttgtgc gttcctgcct aatttttata tcttctaagc aaagtgcctt aggatagctt
2941 gggatgagat gtgtgtgaaa gtatgtacaa gagaaaacgg aagagagagg aaatgaggtg
3001 gggttggagg aaacccatgg ggacagattc ccattcttag cctaacgttc gtcattgcct
3061 cgtcacatca atgcaaaagg tcctgatttt gttccagcaa aacacagtgc aatgttctca
3121 gagtgacttt cgaaataaat tgggcccaag agctttaact cggtcttaaa atatgcccaa
3181 atttttactt tgtttttctt ttaataggct gggccacatg ttggaaataa gctagtaatg
3241 ttgtttttctg tcaatattga atgtgatggt acagtaaacc aaaacccaac aatgtggcca
3301 gaaagaaaga gcaataataa ttaattcaca caccatatgg attctattta taaatcaccc
3361 acaaacttgt tctttaattt catcccaatc acttttttcag aggcctgtta tcatagaagt
```

Figure 10 (cont.)

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3421 cattttagac tctcaatttt aaattaattt tgaatcacta atattttcac agtttattaa
3481 tatatttaat ttctatttaa attttagatt atttttatta ccatgtactg aatttttaca
3541 tcctgatacc ctttccttct ccatgtcagt atcatgttct ctaattatct tgccaaattt
3601 tgaaactaca cacaaaaagc atacttgcat tatttataat aaaattgcat tcagtggctt
3661 tttaaaaaaa atgtttgatt caaaacttta acatactgat aagtaagaaa caattataat
3721 ttctttacat actcaaaacc aagatagaaa aaggtgctat cgttcaactt caaaacatgt
3781 ttcctagtat taaggacttt aatatagcaa cagacaaaat tattgttaac atggatgtta
3841 cagctcaaaa gatttataaa agattttaac ctatttttctc ccttattatc cactgctaat
3901 gtggatgtat gttcaaacac cttttagtat tgatagctta catatggcca aaggaataca
3961 gtttatagca aaacatgggt atgctgtagc taactttata aaagtgtaat ataacaatgt
4021 aaaaaattat atatctggga ggatttttttg gttgcctaaa gtggctatag ttactgattt
4081 tttattatgt aagcaaaacc aataaaaatt taagtttttt taacaactac cttatttttc
4141 actgtacaga cactaattca ttaaatacta attgattgtt taaaagaaat ataaatgtga
4201 caagtggaca ttatttatgt taaatataca attatcaagc aagtatgaag ttattcaatt
4261 aaaatgccac atttctggtc tctggg
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SEQ ID NO:16

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 121 cgcgatgctg cccggtttgg cactgctcct gctggccgcc tggacggctc gggcgctgga
 181 ggtacccact gatggtaatg ctggcctgct ggctgaaccc cagattgcca tgttctgtgg
 241 cagactgaac atgcacatga atgtccagaa tgggaagtgg gattcagatc catcagggac
 301 caaaacctgc attgatacca aggaaggcat cctgcagtat tgccaagaag tctaccctga
 361 actgcagatc accaatgtgg tagaagccaa ccaaccagtg accatccaga actggtgcaa
 421 gcggggccgc aagcagtgca agacccatcc ccactttgtg attccctacc gctgcttagt
 481 tggtgagttt gtaagtgatg ccccttctcgt tcctgacaag tgcaaattct tacaccagga
 541 gaggatggat gtttgcgaaa ctcatcttca ctggcacacc gtcgccaaag agacatgcag
 601 tgagaagagt accaacttgc atgactacgg catgttgctg ccctgcggaa ttgacaagtt
 661 ccgaggggta gagtttgtgt gttgcccact ggctgaagaa agtgacaatg tggattctgc
 721 tgatgcggag gaggatgact cggatgtctg gtggggcgga gcagacacag actatgcaga
 781 tgggagtgaa gacaaagtag tagaagtagc agaggaggaa gaagtggctg aggtggaaga
 841 agaagaagcc gatgatgacg aggacgatga ggatggtgat gaggtagagg aagaggctga
 901 ggaaccctac gaagaagcca cagagagaac caccagcatt gccaccacca ccaccaccac
 961 cacagagtct gtggaagagg tggttcgaga ggtgtgctct gaacaagccg agacggggcc
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1081 cttttacggc ggatgtggcg gcaaccggaa caactttgac acagaagagt actgcatggc
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1201 tctcgagaca cctggggatg agaatgaaca tgcccatttc cagaaagcca aagagaggct
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1381 agtggaatct ttggaacagg aagcagccaa cgagagacag cagctggtgg agacacacat
1441 ggccagagtg gaagccatgc tcaatgaccg ccgccgcctg gccctggaga actacatcac
1501 cgctctgcag gctgttcctc ctcggcctcg tcacgtgttc aatatgctaa agaagtatgt
1561 ccgcgcagaa cagaaggaca gacagcacac cctaaagcat ttcgagcatg tgcgcatggt
1621 ggatcccaag aaagccgctc agatccggtc ccaggttatg acacacctcc gtgtgattta
1681 tgagcgcatg aatcagtctc tctccctgct ctacaacgtg cctgcagtgg ccgaggagat
1741 tcaggatgaa gttgatgagc tgcttcagaa agagcaaaac tattcagatg acgtcttggc
1801 caacatgatt agtgaaccaa ggatcagtta cggaaacgat gctctcatgc catctttgac
1861 cgaaacgaaa accaccgtgg agctccttcc cgtgaatgga gagttcagcc tggacgatct
1921 ccagccgtgg cattcttttg gggctgactc tgtgccagcc aacacagaaa acgaagttga
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2041 gacaaatatc aagacggagg agatctctga agtgaagatg gatgcagaat tccgacatga
2101 ctcaggatat gaagttcatc atcaaaaatt ggtgttcttt gcagaagatg tgggttcaaa
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Figure 10 (cont.)

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2341 aaatccaacc tacaagttct ttgagcagat gcagaactag acccccgcca cagcagcctc
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2521 aacacaagta gatgcctgaa cttgaattaa tccacacatc agtaatgtat tctatctctc
2581 tttacatttt ggtctctata ctacattatt aatgggtttt gtgtactgta aagaatttag
2641 ctgtatcaaa ctagtgcatg aatagattct ctcctgatta tttatcacat agccccttag
2701 ccagttgtat attattcttg tggtttgtga cccaattaag tcctacttta catatgcttt
2761 aagaatcgat gggggatgct tcatgtgaac gtgggagttc agctgcttct cttgcctaag
2821 tattcctttc ctgatcacta tgcattttaa agttaaacat ttttaagtat ttcagatgct
2881 ttagagagat tttttttcca tgactgcatt ttactgtaca gattgctgct tctgctatat
2941 ttgtgatata ggaattaaga ggatacacac gtttgtttct tcgtgcctgt tttatgtgca
3001 cacattaggc attgagactt caagcttttc ttttttgtc cacgtatctt tgggtctttg
3061 ataaagaaaa gaatccctgt tcattgtaag cacttttacg gggcgggtgg ggaggggtgc
3121 tctgctggtc ttcaattacc aagaattc
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SEQ ID NO:17

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 121 tgtggcacca accgcattcc agagtctggc ggagacaaca gcgtgtttga catctttgaa
 181 ctcaccgggg ccgcccgcaa ggggtctggg cgccgactgg tgaagggccc cgaccttcc
 241 agcccagctt tccgcatcga ggatgccaac ctgatccccc ctgtgcctga tgacaagttc
 301 caagacctgg tggatgctgt gcggacagaa aagggtttcc tccttctggc atccctgagg
 361 cagatgaaga agacccgggg cacgctgctg gccctggagc ggaaagacca ctctggccag
 421 gtcttcagcg tggtgtccaa tggcaaggcg ggcaccctgg acctcagcct gaccgtccaa
 481 ggaaagcagc acgtggtgtc tgtggaagaa gctctcctgg caaccggcca gtggaagagc
 541 atcaccctgt ttgtgcagga agacagggcc cagctgtaca tcgactgtga aaagatggag
 601 aatgctgagt tggacgtccc catccaaagc gtcttcacca gagacctggc cagcatcgcc
 661 agactccgca tcgcaaaggg gggcgtcaat gacaatttcc aggggtgct gcagaatgtg
 721 aggtttgtct ttggaaccac accagaagac atcctcagga acaaaggctg ctccagctct
 781 accagtgtcc tcctcaccct tgacaacaac gtggtgaatg gttccagccc tgccatccgc
 841 actaactaca ttggccacaa gacaaaggac ttgcaagcca tctgcggcat ctcctgtgat
 901 gagctgtcca gcatggtcct ggaactcagg ggcctgcgca ccattgtgac cacgctgcag
 961 gacagcatcc gcaaagtgac tgaagagaac aaagagttgg ccaatgagct gaggcggcct
1021 cccctatgct atcacaacgg agttcagtac agaaataacg aggaatggac tgttgatagc
1081 tgcactgagt gtcactgtca gaactcagtt accatctgca aaaaggtgtc ctgccccatc
1141 atgccctgct ccaatgccac agttcctgat ggagaatgct gtcctcgctg ttggcccagc
1201 gactctgcgg acgatggctg gtctccatgg tccgagtgga cctcctgttc tacgagctgt
1261 ggcaatggaa ttcagcagcg cggccgctcc tgcgatagcc tcaacaaccg atgtgagggc
1321 tcctcggtcc agacacggac ctgccacatt caggagtgtg acaagagatt taaacaggat
1381 ggtggctgga gccactggtc cccgtggtca tcttgttctg tgacatgtgg tgatggtgtg
1441 atcacaagga tccggctctg caactctccc agcccccaga tgaacgggaa accctgtgaa
1501 ggcgaagcgc gggagaccaa agcctgcaag aaagacgcct gccccatcaa tggaggctgg
1561 ggtccttggt caccatggga catctgttct gtcacctgtg gaggaggggt acagaaacgt
1621 agtcgtctct gcaacaaccc cacaccccag tttggaggca aggactgcgt tggtgatgta
1681 acagaaaacc agatctgcaa caagcaggac tgtccaattg atggatgcct gtccaatccc
1741 tgctttgccg gcgtgaagtg tactagctac cctgatggca gctggaaatg tggtgcttgt
1801 ccccctggtt acagtggaaa tggcatccag tgcacagatg ttgatgagtg caaagaagtg
1861 cctgatgcct gcttcaacca caatggagag caccggtgtg agaacacgga ccccggctac
1921 aactgcctgc cctgcccccc acgcttcacc ggctcacagc ccttcggcca gggtgtcgaa
1981 catgccacgg ccaacaaaca ggtgtgcaag ccccgtaacc cctgcacgga tgggacccac
2041 gactgcaaca agaacgccaa gtgcaactac ctgggccact atagcgaccc catgtaccgc
2101 tgcgagtgca agcctggcta cgctggcaat ggcatcatct gcggggagga cacagacctg
```

Figure 10 (cont.)

```
2161 gatggctggc ccaatgagaa cctggtgtgc gtggccaatg cgacttacca ctgcaaaaag
2221 gataattgcc ccaaccttcc caactcaggg caggaagact atgacaagga tggaattggt
2281 gatgcctgtg atgatgacga tgacaatgat aaaattccag atgacaggga caactgtcca
2341 ttccattaca acccagctca gtatgactat gacagagatg atgtgggaga ccgctgtgac
2401 aactgtccct acaaccacaa cccagatcag gcagacacag acaacaatgg ggaaggagac
2461 gcctgtgctg cagacattga tggagacggt atcctcaatg aacgggacaa ctgccagtac
2521 gtctacaatg tggaccagag agacactgat atggatgggg ttggagatca gtgtgacaat
2581 tgccccttgg aacacaatcc ggatcagctg gactctgact cagaccgcat tggagatacc
2641 tgtgacaaca atcaggatat tgatgaagat ggccaccaga acaatctgga caactgtccc
2701 tatgtgccca atgccaacca ggctgaccat gacaaagatg gcaagggaga tgcctgtgac
2761 cacgatgatg acaacgatgg cattcctgat gacaaggaca actgcagact cgtgcccaat
2821 cccgaccaga aggactctga cggcgatggt cgaggtgatg cctgcaaaga tgattttgac
2881 catgacagtg tgccagacat cgatgacatc tgtcctgaga atgttgacat cagtgagacc
2941 gatttccgcc gattccagat gattcctctg gaccccaaag ggacatccca aaatgaccct
3001 aactgggttg tacgccatca gggtaaagaa ctcgtccaga ctgtcaactg tgatcctgga
3061 ctcgctgtag gttatgatga gtttaatgct gtggacttca gtggcacctt cttcatcaac
3121 accgaaaggg acgatgacta tgctggattt gtctttggct accagtccag cagccgcttt
3181 tatgttgtga tgtggaagca agtcacccag tcctactggg acaccaaccc cacgagggct
3241 cagggatact cgggcctttc tgtgaaagtt gtaaactcca ccacagggcc tggcgagcac
3301 ctgcggaacg ccctgtggca cacaggaaac acccctggcc aggtgcgcac cctgtggcat
3361 gaccctcgtc acataggctg gaaagatttc accgcctaca gatggcgtct cagccacagg
3421 ccaaagacgg gtttcattag agtggtgatg tatgaaggga agaaaatcat ggctgactca
3481 ggacccatct atgataaaac ctatgctggt ggtagactag ggttgtttgt cttctctcaa
3541 gaaatggtgt tcttctctga cctgaaatac gaatgtagag atccctaatc atcaaattgt
3601 tgattgaaag actgatcata aaccaatgct ggtattgcac cttctggaac tatgggcttg
3661 agaaaacccc caggatcact tctccttggc ttccttcttt tctgtgcttg catcagtgtg
3721 gactcctaga acgtgcgacc tgcctcaaga aaatgcagtt ttcaaaaaca gactcagcat
3781 tcagcctcca atgaataaga catcttccaa gcatataaac aattgctttg gtttcctttt
3841 gaaaaagcat ctacttgctt cagttgggaa ggtgcccatt ccactctgcc tttgtcacag
3901 agcagggtgc tattgtgagg ccatctctga gcagtggact caaaagcatt ttcaggcatg
3961 tcagagaagg gaggactcac tagaattagc aaacaaaacc accctgacat cctccttcag
4021 gaacacgggg agcagaggcc aaagcactaa ggggagggcg catacccgag acgattgtat
4081 gaagaaaata tggaggaact gttacatgtt cggtactaag tcattttcag gggattgaaa
4141 gactattgct ggatttcatg atgctgactg gcgttagctg attaacccat gtaaataggc
4201 acttaaatag aagcaggaaa gggagacaaa gactggcttc tggacttcct ccctgatccc
4261 caccttact catcacctgc agtggccaga attagggaat cagaatcgaa accagtgtaa
4321 ggcagtgctg gctgccattg cctggtcaca ttgaaattgg tggcttcatt ctagatgtag
4381 cttgtgcaga tgtagcagga aaataggaaa acctaccatc tcagtgagca ccag
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SEQ ID NO:18

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   1 atttctcttt agttctttgc aagaaggtag agataaagac acttttttcaa aaatggcaat
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 121 aactgtgaag tcatccaaag gtggtcccgg atcagcggtg agcccctatc ctaccttcaa
 181 tccatcctcg gatgtcgctg ccttgcataa ggccataatg gttaaaggtg tggatgaagc
 241 aaccatcatt gacattctaa ctaagcgaaa caatgcacag cgtcaacaga tcaaagcagc
 301 atatctccag gaaacaggaa agcccctgga tgaaacactg aagaaagccc ttacaggtca
 361 ccttgaggag gttgttttag ctctgctaaa aactccagcg caatttgatg ctgatgaact
 421 tcgtgctgcc atgaagggcc ttggaactga tgaagatact ctaattgaga ttttggcatc
 481 aagaactaac aaagaaatca gagacattaa cagggtctac agagaggaac tgaagagaga
 541 tctggccaaa gacataacct cagacacatc tggagatttt cggaacgctt tgctttctct
 601 tgctaagggt gaccgatctg aggactttgg tgtgaatgaa gacttggctg attcagatgc
 661 cagggccttg tatgaagcag gagaaaggag aaaggggaca gacgtaaacg tgttcaatac
 721 catccttacc accagaagct atccacaact tcgcagagtg tttcagaaat acaccaagta
 781 cagtaagcat gacatgaaca aagttctgga cctggagttg aaaggtgaca ttgagaaatg
```

Figure 10 (cont.)

```
 841 cctcacagct atcgtgaagt gcgccacaag caaaccagct ttctttgcag agaagcttca
 901 tcaagccatg aaaggtgttg gaactcgcca taaggcattg atcaggatta tggtttcccg
 961 ttctgaaatt gacatgaatg atatcaaagc attctatcag aagatgtatg gtatctccct
1021 ttgccaagcc atcctggatg aaaccaaagg agattatgag aaaatcctgg tggctctttg
1081 tggaggaaac taaacattcc cttgatggtc tcaagctatg atcagaagac tttaattata
1141 tattttcatc ctataagctt aaataggaaa gtttcttcaa caggattaca gtgtagctac
1201 ctacatgctg aaaaatatag cctttaaatc atttttatat tataactctg tataatagag
1261 ataagtccat tttttaaaaa tgttttcccc aaaccataaa accctataca agttgttcta
1321 gtaacaatac atgagaaaga tgtctatgta gctgaaaata aaatgacgtc acaagac
```

SEQ ID NO:19

```
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  61 actgtcccag gtcccaggtc ccggcccgga gctatggagc ggcgctggcc cctggggcta
 121 gggctggtgc tgctgctctg cgccccgctg ccccgggggg cgcgcgccaa ggaagttact
 181 ctgatggaca caagcaaggc acagggagag ctgggctggc tgctggatcc cccaaaagat
 241 gggtggagtg aacagcaaca gatactgaat gggacacccc tctacatgta ccaggactgc
 301 ccaatgcaag gacgcagaga cactgaccac tggcttcgct ccaattggat ctaccgcggg
 361 gaggaggctt cccgcgtcca cgtggagctg cagttcaccg tgcgggactg caagagtttc
 421 cctgggggag ccgggcctct gggctgcaag gagaccttca accttctgta catggagagt
 481 gaccaggatg tgggcattca gctccgacgg cccttgttcc agaaggtaac cacggtggct
 541 gcagaccaga gcttcaccat tcgagacctt gcgtctggct ccgtgaagct gaatgtggag
 601 cgctgctctc tgggccgcct gacccgccgt ggcctctacc tcgctttcca caacccgggt
 661 gcctgtgtgg ccctggtgtc tgtccgggtc ttctaccagc gctgtcctga gaccctgaat
 721 ggcttggccc aattcccaga cactctgcct ggccccgctg ggttggtgga agtggcgggc
 781 acctgcttgc cccacgcgcg ggccagcccc aggccctcag gtgcaccccg catgcactgc
 841 agccctgatg gcgagtggct ggtgcctgta ggacggtgcc actgtgagcc tggctatgag
 901 gaaggtggca gtggcgaagc atgtgttgcc tgccctagcg gctcctaccg gatggacatg
 961 gacacacccc attgtctcac gtgcccccag cagagcactg ctgagtctga gggggccacc
1021 atctgtacct gtgagagcgg ccattacaga gctcccgggg agggccccca ggtggcatgc
1081 acaggtcccc cctcggcccc ccgaaacctg agcttctctg cctcagggac tcagctctcc
1141 ctgcgttggg aacccccagc agatacgggg ggacgccagg atgtcagata cagtgtgagg
1201 tgttcccagt gtcagggcac agcacaggac ggggggccct gccagccctg tggggtgggc
1261 gtgcacttct cgccgggggc ccgggcgctc accacacctg cagtgcatgt caatggcctt
1321 gaaccttatg ccaactacac ctttaatgtg gaagcccaaa atggagtgtc agggctgggc
1381 agctctggcc atgccagcac ctcagtcagc atcagcatgg ggcatgcaga gtcactgtca
1441 ggcctgtctc tgagactggt gaagaaagaa ccgaggcaac tagagctgac ctgggcgggg
1501 tcccggcccc gaagccctgg ggcgaacctg acctatgagc tgcacgtgct gaaccaggat
1561 gaagaacggt accagatggt tctagaaccc agggtcttgc tgacagagct gcagcctgac
1621 accacataca tcgtcagagt ccgaatgctg accccactgg gtcctggccc tttctccccc
1681 gatcatgagt ttcggaccag cccaccagtg tccaggggcc tgactggagg agagattgta
1741 gccgtcatct ttgggctgct gcttggtgca gccttgctgc ttgggattct cgttttccgg
1801 tccaggagag cccagcggca gaggcagcag aggcacgtga ccgcgccacc gatgtggatc
1861 gagaggacaa gctgtgctga agccttatgt ggtacctcca ggcatacgag gaccctgcac
1921 agggagcctt ggactttacc cggaggctgg tctaattttc cttcccggga gcttgatcca
1981 gcgtggctga tggtggacac tgtcatagga gaaggagagt ttggggaagt gtatcgaggg
2041 accctcaggc tccccagcca ggactgcaag actgtggcca ttaagacctt aaaagacaca
2101 tccccaggtg gccagtggtg gaacttcctt cgagaggcaa ctatcatggg ccagtttagc
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2221 acagaattta tggagaatgc agccctggat gccttcctga gggagcggga ggaccagctg
2281 gtccctgggc agctagtggc catgctgcag ggcatagcat ctggcatgaa ctacctcagt
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2401 tgctgcaagg tgtctgactt tggcctgact cgcctcctgg atgactttga tggcacatac
2461 gaaacccagg gaggaaagat ccctatccgt tggacagccc ctgaagccat tgcccatcgg
2521 atcttcacca cagccagcga tgtgtggagc tttgggattg tgatgtggga ggtgctgagc
```

Figure 10 (cont.)

```
2581 tttggggaca agccttatgg ggagatgagc aatcaggagg ttatgaagag cattgaggat
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2701 tgctgggcat atgaccgtgc ccgccggcca cacttccaga agcttcaggc acatctggag
2761 caactgcttg ccaaccccca ctccctgcgg accattgcca actttgaccc cagggtgact
2821 cttcgcctgc ccagcctgag tggctcagat gggatcccgt atcgaaccgt ctctgagtgg
2881 ctcgagtcca tacgcatgaa acgctacatc ctgcacttcc actcggctgg gctggacacc
2941 atggagtgtg tgctggagct gaccgctgag gacctgacgc agatgggaat cacactgccc
3001 gggcaccaga agcgcattct ttgcagtatt cagggattca aggactgatc cctcctctca
3061 ccccatgccc aatcagggtg caaggagcaa ggacggggcc aaggtcgctc atggtcactc
3121 cctgcgcccc ttcccacaac ctgccagact aggctatcgg tgctgcttct gcccgcttta
3181 aggagaaccc tgctctgcac cccagaaaac ctctttgttt taaaagggag gtgggggtag
3241 aagtaaaagg atgatcatgg gagggagctc aggggttaat atatatacat acatacacat
3301 atatatattg ttgtaaataa acaggaaatg attttctgcc tccatcccac ccatcagggc
3361 tgcaggcact
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SEQ ID NO:20

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  61 ccgcagtggc gtcctggagc cctcctcagt gctgaagctg ctgaaagatg gcagaagaag
 121 tggtggtagt agccaaattt gattatgtgg cccaacaaga acaagagttg gacatcaaga
 181 agaatgagag attatggctt ctggatgatt ctaagtcctg gtggcgagtt cgaaattcca
 241 tgaataaaac aggttttgtg ccttctaact atgtggaaag gaaaaacagt gctcggaaag
 301 catctattgt gaaaaaccta aaggatacct taggcattgg aaaagtgaaa agaaaaccta
 361 gtgtgccaga ttctgcatct cctgctgatg atagttttgt tgacccaggg gaacgtctct
 421 atgacctcaa catgcccgct tatgtgaaat ttaactacat ggctgagaga gaggatgaat
 481 tatcattgat aaaggggaca aaggtgatcg tcatggagaa atgcagtgat gggtggtggc
 541 gtggtagcta caatggacaa gttggatggt tcccttcaaa ctatgtaact gaagaaggtg
 601 acagtccttt gggtgaccat gtgggttctc tgtcagagaa attagcagca gtcgtcaata
 661 acctaaatac tgggcaagtg ttgcatgtgg tacaggctct ttacccattc agctcatcta
 721 atgatgaaga acttaatttc gagaaaggag atgtaatgga tgttattgaa aaacctgaaa
 781 atgacccaga gtggtggaaa tgcaggaaga tcaatggtat ggttggtcta gtaccaaaaa
 841 actatgttac cgttatgcag aataatccat taacttcagg tttggaacca tcacctccac
 901 agtgtgatta cattaggcct tcactcactg gaaagtttgc tggcaatcct tggtattatg
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1081 ggaaaaacaa gcattttaaa gtccaactaa aagagactgt ctactgcatt gggcagcgta
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1201 aacaaggaga aaaattatat cttgtcaagc atttatcatg atactgctga ccagaagtga
1261 ctgctgtgta gctgtaattt gtcatgtaat tgaagactga gaaaatgttg ggtccagtcg
1321 tgcttgattg gaaattgttg tttctaaatc tatatgagaa ttgacaataa gtatttttat
1381 tataactcag cccatacata tatactatgt atgcagtgca tctgcataga acagttcctt
1441 atccttggcc ttctgtttta ttgtttttt ctttgctgtt ttccctttgc ttctaatatt
1501 acagttttgt attttgtaaa caaaaatcaa ataatgcata tcagaatctt tatatggaag
1561 aaatccttta ttgcctttcc tttgtttcct tgtaaaggca ccctgttctg ttatggtttt
1621 tcattatata aaattattat atctatatat gacatatgct aaaatttctt ggagagtgtt
1681 aatcttttct gtgactaaat agcaataata agtggaaaat tagaaattat ttccaggtat
1741 tatatttgtc acaggccatt gtaaatacca agtatattgt gtctgccata atttttaaaa
1801 atacattcat tgtcttcagt catacagcaa gacacatgag acatagatta gaaaacatgt
1861 tgtacaattt taatttacaa ctgttggaaa taaaaatcac ttaatttttt tcc
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SEQ ID NO:21

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 121 aagtcccgtt gtatcagagt aagatggacg gtagctttga ttgtgattgt ggtgagctgg
 181 agccacctga tcactaacaa aagacatctt ctgttaacca acagccgcca gggcttcctg
```

Figure 10 (cont.)

```
 241 ttgaaataaa tatatagcaa caaaggaaaa aaagaagcaa aacggaaata gtgcttacca
 301 gcaccttaga atgatgctgc tcaggaccag tccaacactg aatgtatctg cactgtgagg
 361 agaatgttca tagaagcctg ttgtgtgcat atttattcac atttttgtta aatgttaaat
 421 cgtttagcac ggtaatctga gtgcacagta tgtcatttca ttccgtttga gttcttgtt
 481 ttcgttaaat gtctgcagag ttgctgcccc tttcttgaac tatgagtact gcaatctttt
 541 taattctcaa tatgaataga gctttttgag ctttaaatct aaggggaact cgacaggcct
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 661 ttctcccttt ttgaaagatc tttccttttg atgccagttt tcttccttgt ttacacaagt
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1081 aggagtacat ggagacagac ttggctaatg tgctggagca gggcccttta ctggaagagc
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1261 agataggtga ctttggtctt gcacggatca tggatcctca ttattcccat aagggtcatc
1321 tttctgaagg attggttact aaatggtaca gatctccacg tcttttactt tctcctaata
1381 attatactaa agccattgac atgtgggctg caggctgcat ctttgctgaa atgctgactg
1441 gtaaaaccct ttttgcaggt gcacatgaac ttgaacagat gcagctgatt ttagaatcta
1501 ttcctgttgt acatgaggaa gatcgtcagg agcttctcag cgtaattcca gtttacatta
1561 gaaatgacat gactgagcca cacaaacctt taactcagct gcttccagga attagtcgag
1621 aagcactgga tttcctggaa caaattttga catttagccc catggatcgg ttaacagcag
1681 aagaagcact ctcccatcct tacatgagca tatattcttt tccaatggat gagccaattt
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1861 ctgtacataa caactttgat attgatgaag ttcagcttga tccaagagct ctgtccgatg
1921 tcactgatga agaagaagta caagttgatc cccgaaaata tttggatgga gatcgggaaa
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2461 agtcagtaag ccaagaaaaa caggaaaaag gaatggcaaa tctggctcaa ttagaagcct
2521 tgtaccagtc ttcttgggac agccagtttg tgagtggtgg ggaggactgt tttttcataa
2581 atcagttttg tgaggtaagg aaggatgaac aagttgagaa ggaaaacact tacactagtt
2641 acttggacaa gttctttagc aggaaagaag atactgaaat gctagaaact gagccagtag
2701 aggatgggaa gcttggggag agaggacatg aggaaggatt tctgaacaac agtggggagt
2761 tcctctttaa caagcagctc gagtccatag gcatcccaca gtttcacagt ccagttgggt
2821 caccacttaa gtcaatacag gccacattaa caccttctgc tatgaaatct tcccctcaaa
2881 ttcctcatca aacatacagc agcattctga aacatctgaa ctaaaacact cagcagacat
2941 ttatctttgt attcttcatg aaatgtgttt tgtctttttt tattactagt gtttaagtca
3001 ttttttactt gaatcagatg gtgtcattta gtaaggattt tatgagttct tgtttttaa
3061 aatccagact ttctttttct acatgtgaga tagttttcat tttaactggc atgtcatttg
3121 cacacaaaaa taaagactag agcaaaataa tgcaacgcag gaggagaaaa gaaatgcact
3181 aagacaagaa cattctctca tagaacattg atctgtttta caggaaacaa accttgcctt
3241 gaaatttaca cagtgag
```

SEQ ID NO:22

```
   1 ggtctttgag cgctaacgtc tttctgtctc cccgcggtgg tgatgacggt gaaaactgag
```

Figure 10 (cont.)

```
  61 gctgctaagg gcaccctcac ttactccagg atgaggggca tggtggcaat tctcatcgct
 121 ttcatgaagc agaggaggat gggtctgaac gactttattc agaagattgc caataactcc
 181 tatgcatgca aacaccctga agttcagtcc atcttgaaga tctcccaacc tcaggagcct
 241 gagcttatga atgccaaccc ttctcctcca ccaagtcctt ctcagcaaat caaccttggc
 301 ccgtcgtcca atcctcatgc taaaccatct gactttcact tcttgaaagt gatcggaaag
 361 ggcagttttg gaaaggttct tctagcaaga cacaaggcag aagaagtgtt ctatgcagtc
 421 aaagttttac agaagaaagc aatcctgaaa aagaaagagg agaagcatat tatgtcggag
 481 cggaatgttc tgttgaagaa tgtgaagcac cctttcctgg tgggccttca cttctctttc
 541 cagactgctg acaaattgta ctttgtccta gactacatta atggtggaga gttgttctac
 601 catctccaga gggaacgctg cttcctggaa ccacgggctc gtttctatgc tgctgaaata
 661 gccagtgcct tgggctacct gcattcactg aacatcgttt atagagactt aaaaccagag
 721 aatattttgc tagattcaca gggacacatt gtccttactg acttcggact ctgcaaggag
 781 aacattgaac acaacagcac aacatccacc ttctgtggca cgccggagta tctcgcacct
 841 gaggtgcttc ataagcagcc ttatgacagg actgtggact ggtggtgcct gggagctgtc
 901 ttgtatgaga tgctgtatgg cctgccgcct ttttatagcc gaaacacagc tgaaatgtac
 961 gacaacattc tgaacaagcc tctccagctg aaaccaaata ttacaaattc cgcaagacac
1021 ctcctggagg gcctcctgca gaaggacagg acaaagcggc tcggggccaa ggatgacttc
1081 atggagatta agagtcatgt cttcttctcc ttaattaact gggatgatct cattaataag
1141 aagattactc ccccttttaa cccaaatgtg agtgggccca acgacctacg gcactttgac
1201 cccgagttta ccgaagagcc tgtccccaac tccattggca agtcccctga cagcgtcctc
1261 gtcacagcca gcgtcaagga agctgccgag gctttcctag gcttttccta tgcgcctccc
1321 acggactctt tcctctgaac cctgttaggg cttggtttta aaggatttta tgtgtgtttc
1381 cgaatgtttt agttagcctt ttggtggagc cgccagctga caggacatct tacaagagaa
1441 tttgcacatc tctggaagct tagcaatctt attgcacact gttcgctgga agcttttga
1501 agagcacatt ctcctcagtg agctcatgag gttttcattt ttattcttcc ttccaacgtg
1561 gtgctatctc tgaaacgagc gttagagtgc cgccttagac ggaggcagga gtttcgttag
1621 aaagcggacg ctgttctaaa aaaggtctcc tgcagatctg tctgggctgt gatgacgaat
1681 attatgaaat gtgccttttc tgaagagatt gtgttagctc caaagctttt cctatcgcag
1741 tgtttcagtt ctttattttc ccttgtggat atgctgtgtg aaccgtcgtg tgagtgtggt
1801 atgcctgatc acagatggat tttgttataa gcatcaatgt gacacttgca ggacactaca
1861 acgtgggaca ttgtttgttt cttccatatt tggaagataa atttatgtgt agacttttt
1921 gtaagatacg gttaataact aaaatttatt gaaatggtct tgcaatgact cgtattcaga
1981 tgcttaaaga aagcattgct gctacaaata tttctatttt tagaaagggt ttttatggac
2041 caatgcccca gttgtcagtc agagccgttg gtgtttttca ttgtttaaaa tgtcacctgt
2101 aaaatgggca ttatttatgt ttttttttt gcattcctga taattgtatg tattgtataa
2161 agaacgtctg tacattgggt tataacacta gtatatttaa acttacaggc ttatttgtaa
2221 tgtaaaccac cattttaatg tactgtaatt aacatggtta taatacgtac aatccttccc
2281 tcatcccatc acacaacttt ttttgtgtgt gataaactga ttttggtttg caataaaacc
2341 ttgaaaaata ttta
```

SEQ ID NO:23

```
1 gagcagcaga atttcaactc cagtagactt gaatatgcct ctgggcaaag aagcagagct
  61 aacgaggaaa gggatttaaa gagtttttct tgggtgtttg tcaaactttt attccctgtc
 121 tgtgtgcaga ggggattcaa cttcaatttt tctgcagtgg ctctgggtcc agccccttac
 181 ttaaagatct ggaaagcatg aagactgggc tttttttcct atgtctcttg ggaactgcag
 241 ctgcaatccc gacaaatgca agattattat ctgatcattc caaaccaact gctgaaacgg
 301 tagcacctga caacactgca atccccagtt taagggctga agctgaagaa aatgaaaaag
 361 aaacagcagt atccacagaa gacgattccc accataaggc tgaaaaatca tcagtactaa
 421 agtcaaaaga ggaaagccat gaacagtcag cagaacaggg caagagttct agccaagagc
 481 tgggattgaa ggatcaagag gacagtgatg gtcacttaag tgtgaatttg gagtatgcac
 541 caactgaagg tacattggac ataaaagaag atatgagtga gcctcaggag aaaaaactct
 601 cagagaacac tgatttttg gctcctggtg ttagttcctt cacagattct aaccaacaag
 661 aaagtatcac aaagagagag gaaaaccaag aacaacctag aaattattca catcatcagt
 721 tgaacaggag cagtaaacat agccaaggcc taagggatca aggaaaccaa gagcaggatc
```

Figure 10 (cont.)

```
 781 caaatatttc caatggagaa gaggaagaag aaaaagagcc aggtgaagtt ggtacccaca
 841 atgataacca agaaagaaag acagaattgc ccagggagca tgctaacagc aagcaggagg
 901 aagacaatac ccaatctgat gatattttgg aagagtctga tcaaccaact caagtaagca
 961 agatgcagga ggatgaattt gatcagggta accaagaaca agaagataac tccaatgcag
1021 aaatggaaga ggaaaatgca tcgaacgtca ataagcacat tcaagaaact gaatggcaga
1081 gtcaagaggg taaaactggc ctagaagcta tcagcaacca caaagagaca gaagaaaaga
1141 ctgtttctga ggctctgctc atggaaccta ctgatgatgg taataccacg cccagaaatc
1201 atggagttga tgatgatggc gatgatgatg gcgatgatgg cggcactgat ggccccaggc
1261 acagtgcaag tgatgactac ttcatcccaa gccaggcctt tctggaggcc gagagagctc
1321 aatccattgc ctatcacctc aaaattgagg agcaaagaga aaaagtacat gaaaatgaaa
1381 atataggtac cactgagcct ggagagcacc aagaggccaa gaaagcagag aactcatcaa
1441 atgaggagga aacgtcaagt gaaggcaaca tgagggtgca tgctgtggat tcttgcatga
1501 gcttccagtg taaaagaggc cacatctgta aggcagacca acagggaaaa cctcactgtg
1561 tctgccagga tccagtgact tgtcctccaa caaaacccct tgatcaagtt tgtggcactg
1621 acaatcagac ctatgctagt tcctgtcatc tattcgctac taaatgcaga ctggagggga
1681 ccaaaaaggg gcatcaactc cagctggatt attttggagc ctgcaaat
```

SEQ ID NO:24

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   1 cggataagga caaaaaacgc cagaagaaaa gaggcatttt ccccaaagta gcaacaaata
  61 tcatgagagc atggctcttc cagcatctca cacatccgta cccttccgaa gagcagaaga
 121 aacagttagc gcaagacaca ggacttacaa ttctccaagt aaacaactgg tttattaatg
 181 ccagaagaag aatagtacag cccatgattg accagtcaaa tcgagcagtg agccaaggag
 241 cagcatatag tccagagggt cagcccatgg ggagctttgt gttggatggt cagcaacaca
 301 tggggatccg gcctgcagga cctatgagtg gaatgggcat gaatatgggc atggatgggc
 361 aatggcacta catgtaacct tcatcatgta aagcaatcgc aaagcaaggg ggaagtttgc
 421 agagcatgcc aggggactac gtttctcagg gtggtcctat gggaatgagt atggcacagc
 481 caagttacac tcctccccag atgaccccac accctactca attaagacat ggacccccaa
 541 tgcattcata tttgccaagc catccccacc acccagccat gatgatgcac ggaggacccc
 601 ctacccaccc tggaatgact atgtcagcac agagccccac aatgttaaat tctgtagatc
 661 ccaatgttgg cggacaggtt atggacattc atgcccaata gtataaggga actcaaggga
 721 aaaggaaaca cacgcaaaaa ctatttttaag actttctgaa ctttgaccag atgttgacac
 781 ttaatatgaa attccagaca gctgtgatta ttttttactt ttgtcatttt tcatcaagca
 841 acagaggacc aatgcaacaa gaacacaaat gtgaaatcat gggctgactg agacaattct
 901 gtccatgtaa agatcctctg gaaaaagact ccgagagtta taactactgt agtataaata
 961 taggaactaa gttaaacttg tacatttctg ttgatcacgc cgttatgttg cctcaaatag
1021 ttttagaaga gaaaaaaaaa tatatccttg ttttccacac tatgtgtgtt gttcccaaaa
1081 gaatgactgt tttggttcat cagtgaattc accatccagg agagactgtg gtatatattt
1141 taaacctgtt gggccaatga gaaaagaacc acactggaga tcatgatgaa cttttggctg
1201 aacctcatca ctcgaactcc agcttcaaga atgtgttttc atgcccggcc tttgttcctc
1261 cataaatgtg tcctttagtt tcaaacagat ctttatagtt cgtgcttcat aagccaattc
1321 ttattattat ttttgggggga ctcttcttca aagagcttgc caatgaagat ttaaagacag
1381 agcaggagct tcttccagga gttctgagcc ttggttgtgg acaaaacaat cttaagttgg
1441 gcagctttcc tcaacacaaa aaaaagttat taatggtcat tgaaccataa ctaggacttt
1501 atcagaaact caaagcttgg gggataaaaa ggagcaagag aatactgtaa caaacttcgt
1561 acagagttcg gtctattaat tgtttcatgt tagatattct atgtgtttac ctcaattgaa
1621 aaaaaaaaga atgtttttgc tagtatcaga tctgctgtgg aattggtatt gtatgtccat
1681 gaattcttct tttctcagca cgtgttcctc actagaagaa aatgctgtta cctttaagct
1741 ttgtcaaatt tacattaaaa tacttgtatg aggactgtga cgttatgtta aaaaaaaaaa
1801 ggtgttaagt cacaaaaagc ggtaataaat atttcatttt tgattttt
```

SEQ ID NO:25

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1 agcacactga ggaggcgatc cgccagcagg aggtggagca gctggacttc cgagacctcc
  61 tggggaagaa ggtgagtaca aagaccctat cggaagacga cctgaaggag atcccagccg
 121 agcagatgga tttccgtgcc aacctgcagc ggcaagtgaa gccaaagact gtgtctgagg
```

Figure 10 (cont.)

```
 181 aagagaggaa ggtgcacagc ccccagcagg tcgattttcg ctctgtcctg gccaagaagg
 241 ggacttccaa gacccccgtg cctgagaagg tgccaccgcc aaaacctgcc accccggatt
 301 ttcgctcagt gctgggtggc aagaagaaat taccagcaga gaatggcagc agcagtgccg
 361 agaccctgaa tgccaaggca gtggagagtt ccaagcccct gagcaatgca cagccttcag
 421 ggcccttgaa acccgtgggc aacgccaagc ctgctgagac cctgaagcca atgggcaacg
 481 ccaagcctgc cgagaccctg aagcccatgg gcaatgccaa gcctgatgag aacctgaaat
 541 ccgctagcaa agaagaactc aagaaagacg ttaagaatga tgtgaactgc aagagaggcc
 601 atgcagggac cacagataat gaaaagagat cagagagcca ggggacagcc ccagccttca
 661 agcagaagct gcaagatgtt catgtggcag agggcaagaa gctgctgctc cagtgccagg
 721 tgtcttctga ccccccagcc accatcatct ggacgctgaa cggaaagacc atcaagacca
 781 ccaagttcat catcctctcc caggaaggct cactctgctc cgtctccatc gagaaggcac
 841 tgcctgagga cagaggctta tacaagtgtg tagccaagaa tgacgctggc caggcggagt
 901 gctcctgcca agtcaccgtg gatgatgctc cagccagtga gaacaccaag gccccagaga
 961 tgaaatcccg gaggcccaag agctctcttc ctcccgtgct aggaactgag agtgatgcga
1021 ctgtgaaaaa gaaacctgcc cccaagacac ctccgaaggc agcaatgccc cctcagatca
1081 tccagttccc tgaggaccag aaggtacgcg caggagagtc agtggagctg tttggcaaag
1141 tgacaggcac tcagcccatc acctgtacct ggatgaagtt ccgaaagcag atccaggaaa
1201 gcgagcacat gaaggtggag aacagcgaga atggcagcaa gctcaccatc ctggccgcgc
1261 gccaggagca ctgcggctgc tacacactgc tggtggagaa caagctgggc agcaggcagg
1321 cccaggtcaa cctcactgtc gtggataagc cagacccccc agctggcaca ccttgtgcct
1381 ctgacattcg gagctcctca ctgaccctgt cctggtatgg ctcctcatat gatgggggca
1441 gtgctgtaca gtcctacagc atcgagatct gggactcagc caacaagacg tggaaggaac
1501 tagccacatg ccgcagcacc tctttcaacg tccaggacct gctgcctgac cacgaatata
1561 agttccgtgt acgtgcaatc aacgtgtatg gaaccagtga gccaagccag gagtctgaac
1621 tcacaacggt aggagagaaa cctgaagagc cgaaggatga agtggaggtg tcagacgatg
1681 atgagaagga gcccgaggtt gattaccgga cagtgacaat caatactgaa caaaaagtat
1741 ctgacttcta cgacattgag gagagattag gatctgggaa atttggacag gtctttcgac
1801 ttgtagaaaa gaaaactcga aaagtctggg cagggaagtt cttcaaggca tattcagcaa
1861 aagagaaaga gaatatccgg caggagatta gcatcatgaa ctgcctccac caccctaagc
1921 tggtccagtg tgtggatgcc tttgaagaaa aggccaacat cgtcatggtc ctggagatcg
1981 tgtcaggagg ggagctgttt gagcgcatca ttgacgagga ctttgagctg acggagcgtg
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2221 tctttggcac cccagaattt gtggctcctg aagtgatcaa ctatgagccc atcggctacg
2281 ccacagacat gtggagcatc ggggtcatct gctacatcct agtcagtggc ctttcccccc
2341 tcatgggaga caacgataac gaaaccttgg ccaacgttac ctcagccacc tgggacttcg
2401 acgacgaggc attcgatgag atctccgacg atgccaagga tttcatcagc aatctgctga
2461 agaaagatat gaaaaaccgc ctggactgca cgcagtgcct tcagcatcca tggctaatga
2521 aagataccaa gaacatggag gccaagaaac tctccaagga ccggatgaag aagtacatgg
2581 caagaaggaa atggcagaaa acgggcaatg ctgtgagagc cattggaaga ctgtcctcta
2641 tggcaatgat ctcagggctc agtggcagga aatcctcaac agggtcacca accagcccgc
2701 tcaatgcaga aaaactagaa tctgaagaag atgtgtccca agctttcctt gaggctgttg
2761 ctgaggaaaa gcctcatgta aaaccctatt tctctaagac cattcgcgat ttagaagttg
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2941 aggacgggaa ctgctcttta attattagtg atgtttgcgg ggatgacgat gccaagtaca
3001 cctgcaaggc tgtcaacagt cttggagaag ccacctgcac agcagagctc attgtggaaa
3061 cgatggagga aggtgaaggg gaaggggaag aggaagaaga gtgaaacaaa gccagagaaa
3121 agcagtttct aagtcatatt aaaaggacta tttctctaaa actc
```

SEQ ID NO:26

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```

Figure 10 (cont.)

```
 121 ggcgctgctg ggcgcactgt ccgcgggcgc cggggcgcag ccgtaccacg gagagaaggg
 181 catctccgtg ccggaccacg gcttctgcca gcccatctcc atcccgctgt gcacggacat
 241 cgcctacaac cagaccatcc tgcccaacct gctgggccac acgaaccaag aggacgcggg
 301 cctcgaggtg caccagttct acccgctggt gaaggtgcag tgttctcccg aactccgctt
 361 tttcttatgc tccatgtatg cgcccgtgtg caccgtgctc gatcaggcca tcccgccgtg
 421 tcgttctctg tgcgagcgcg cccgccaggg ctgcgaggcg ctcatgaaca agttcggctt
 481 ccagtggccc gagcggctgc gctgcgagaa cttcccggtg cacggtgcgg gcgagatctg
 541 cgtgggccag aacacgtcgg acggctccgg gggcccaggc ggcggcccca ctgcctaccc
 601 taccgcgccc tacctgccgg acctgccctt caccgcgctg cccccggggg cctcagatgg
 661 caggggcgt cccgccttcc ccttctcatg cccccgtcag ctcaaggtgc ccccgtacct
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 781 cggcctgatg tactttaagg aggaggagag gcgcttcgcc cgcctctggg tgggcgtgtg
 841 gtccgtgctg tgctgcgcct cgacgctctt taccgttctc acctacctgg tggacatgcg
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1261 ccaggtagac ggggacctgc tgagcggggt gtgctacgtt ggcctctcca gtgtggacgc
1321 gctgcggggc ttcgtgctgg cgcctctgtt cgtctacctc ttcataggca cgtccttctt
1381 gctggccggc ttcgtgtccc tcttccgtat ccgcaccatc atgaaacacg acggcaccaa
1441 gaccgagaag ctggagaagc tcatggtgcg catcggcgtc ttcagcgtgc tctacacagt
1501 gcccgccacc atcgtcctgg cctgctactt ctacgagcag gccttccgcg agcactggga
1561 gcgcacctgg ctcctgcaga cgtgcaagag ctatgccgtg ccctgcccgc ccggccactt
1621 cccgcccatg agccccgact tcaccgtctt catgatcaag tacctgatga ccatgatcgt
1681 cggcatcacc actggcttct ggatctggtc gggcaagacc ctgcagtcgt ggcgccgctt
1741 ctaccacaga cttagccaca gcagcaaggg ggagactgcg gtatgagccc cggcccctcc
1801 ccacctttcc caccccagcc ctcttgcaag aggagaggca cggtagggaa aagaactgct
1861 gggtgggggc ctgtttctgt aacttttctcc ccctctactg agaagtgacc tggaagtgag
1921 aagttctttg cagatttggg gcgaggggtg atttggaaaa gaagacctgg gtggaaagcg
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2281 ataatctctt ttcactgggg ccaaactgga gcccagatgg gttaatttcc agggtcagac
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2401 tcttgtaaaa taagcatttg gaagtcttgg gaggcctgcc tgctagaatc ctaatgtgag
2461 gatgcaaaag aaatgatgat aacattttga gataaggcca aggagacgtg gagtaggtat
2521 ttttgctact ttttcatttt ctggggaagg caggaggcag aaagacgggt gttttatttg
2581 gtctaatacc ctgaaaagaa gtgatgactt gttgcttttc aaaacaggaa tgcatttttc
2641 cccttgtctt tgttgtaaga gacaaaagag gaaacaaaag tgtctccctg tggaaaggca
2701 taactgtgac gaaagcaact tttataggca aagcagcgca aatctgaggt ttcccgttgg
2761 ttgttaattt ggttgagata aacattcctt tttaaggaaa agtgaagagc agtgtgctgt
2821 cacacaccgt taagccagag gttctgactt cgctaaagga aatgtaagag gttttgttgt
2881 ctgttttaaa taaatttaat tcggaacaca tgatccaaca gactatgtta aaatattcag
2941 ggaaatctct cccttcattt acttttttctt gctataagcc tatatttagg tttcttttct
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3061 taaaggaaag ttaattaaaa aaaaaaagca aagagccatt ttgtcctgtt ttcttggttc
3121 catcaatctg tttattaaac atcatccata tgctgaccct gtctctgtgt ggttgggttg
3181 ggaggcgatc agcagatacc atagtgaacg aagaggaagg tttgaaccat gggccccatc
3241 tttaaagaaa gtcattaaaa gaaggtaaac ttcaaagtga ttctggagtt ctttgaaatg
3301 tgctggaaga cttaaattta ttaatcttaa atcatgtact ttttttctgt aatagaactc
```

Figure 10 (cont.)

```
3361 ggattctttt gcatgatggg gtaaagctta gcagagaatc atgggagcta acctttatcc
3421 cacctttgac actaccctcc aatcttgcaa cactatcctg tttctcagaa cagtttttaa
3481 atgccaatca tagagggtac tgtaaagtgt acaagttact ttatatatgt aatgttcact
3541 tgagtggaac tgctttttac attaaagtta aaatcgatct tgtgtttctt caaccttcaa
3601 aactatctca tctgtcagat ttttaaaact ccaacacagg ttttggcatc ttttgtgctg
3661 tatcttttaa gtgcatgtga aatttgtaaa atagagataa gtacagtatg tatattttgt
3721 aaatctccca tttttgtaag aaaatatata ttgtatttat acatttttac tttggatttt
3781 tgttttgttg gctttaaagg tctaccccac tttatcacat gtacagatca caaataaatt
3841 tttttaaata c
```

SEQ ID NO:27

```
   1 ggggctcggg acggccgggc tgggagctgg agcccacagc gggaagcggc cgccgcccgg
  61 gcctcgcagg gctaggcgag gcgaggggcg gcggggccgg gcgctacggg aaggggaggc
 121 cgcgcggacc gggagccgca ccgcgccagc cgggctgcag cggccgcgca ccaaggctgc
 181 gatggggctg gagacggaga aggcggacgt acagctcttc atggacgacg actcctacag
 241 ccaccacagc ggcctcgagt acgccgaccc cgagaagttc gcggactcgg accaggaccg
 301 ggatccccac cggctcaact cgcatctcaa gctgggcttc gaggatgtga tcgcagagcc
 361 ggtgactacg cactcctttg acaaagtgtg gatctgcagc catgccctct ttgaaatcag
 421 caaatacgta atgtacaagt tcctgacggt gttcctggcc attcccctgg ccttcattgc
 481 gggaattctc tttgccaccc tcagctgtct gcacatctgg attttaatgc cttttgtaaa
 541 gacctgccta atggttctgc cttcagtgca gacaatatgg aagagtgtga cagatgttat
 601 cattgctcca ttgtgtacga gcgtaggacg atgcttctct tctgtcagcc tgcaactgag
 661 ccaggattga atacttggac cccaggtctg gagattggga tactgtaata cttctttgtt
 721 attataacat aaaagcacca ctgttctgtt catttcctag ctgttctaat taagaaaact
 781 attaagatga gcaaccacat ttagaaatgt ttattgacag gtcttttcaa ataatgcttt
 841 tctaattaat agccaaagat ttcatatcta actttgtaac cagaattata cagtaagttg
 901 acaccactta gatttaaagg cagacagttt tgctttagta caatagtata cattttataa
 961 tgatgaactt ataatgatta agggacattt ctataaaaat actacaatag ttttatgcac
1021 aacttcccat taaaaatgag atttcttatt tgtttgtctg tttttactct gggagtaata
1081 ctttttaaat tacctttaca tatatagtca ctggcatact gagaatatac aatgatcctg
1141 gaaattgcag taacaaaagc acacaacgat tatagtaact ataagataca ataaaacaaa
1201 taaatatgaa agtagattca tgaaaatgta ttcctttaaa atattgtttt cctacaggcc
1261 tatttaacaa gatgtttcat tttactgtat attttgtagt taatataaat gttgctctaa
1321 tcagattgct taaaagcatt tttattatat ttatgttgtt gaactaatat atgaaataag
1381 taaatgtagc tcccacaagg taaacttcat tggtaagatt gcactgttct gattatgtaa
1441 gcatttgtac atcttctttg gaaataaaag ataaaa
```

SEQ ID NO:28

```
   1 gtttagaaca gcctacagac ccagtggcac gagacgggcc tctctcccaa acatcttcca
  61 agccagatcc tagtcagtgg gaaagcccca gcttcaaccc ctttgggagc cactctgttc
 121 tgcagaactc cccacccctc tcttctgagg gctcctacca ctttgaccca gataactttg
 181 acgaatccat ggatcccttt aaaccaacta cgaccttaac aagcagtgac ttttgttctc
 241 ccactggtaa tcacgttaat gaaatcttag aatcacccaa gaaggcaaag tcgcgttaa
 301 taacgactac tgaacaagtg aaatttctct gttttctgtt gagtggctgt aaggtgaaga
 361 agcatgaaac tcagtctctc gccctggatg catgttctcg ggatgaaggg gcagtgatct
 421 cccagatttc agacatttct aatagggatg gccatgctac tgatgaggag aaactggcat
 481 ccacgtcatg tggtcagaaa tcagctggtg ccgaggtgaa aggtgagcca gaggaagacc
 541 tggagtactt tgaatgttcc aatgttcctg tgtctaccat aaatcatgcg ttttcatcct
 601 cagaagcagg catagagaag gagacgtgcc agaagatgga agaagacggg tccactgtgc
 661 ttgggctgct ggagtcctct gcagagaagg cccctgtgtc ggtgtcctgt ggaggtgaga
 721 gccccctgga tgggatctgc ctcagcgaat cagacaagac agccgtgctc accttaataa
 781 gagaagagat aattactaaa gagattgaag caaatgaatg gaagaagaaa tacgaagaga
 841 cccggcaaga agttttggag atgaggaaaa ttgtagctga atatgaaaag actattgctc
 901 aaatgattga tgaacaaagg acaagtatga cctctcagaa gagcttccag caactgacca
```

Figure 10 (cont.)

```
 961 tggagaagga acaggccctg gctgacctta actctgtgga aaggtccctt tctgatctct
1021 tcaggagata tgagaacctg aaaggtgttc tggaagggtt caagaagaat gaagaagcct
1081 tgaagaaatg tgctcaggat tacttagcca gagttaaaca agaggagcag cgataccagg
1141 ccctgaaaat ccacgcagaa gagaaactgg acaaagccaa tgaagagatt gctcaggttc
1201 gaacaaaagc aaaggctgag agtgcagctc tccatgctgg actccgcaaa gagcagatga
1261 aggtggagtc cctggaaagg gccctgcagc agaagaacca agaaattgaa gaactgacaa
1321 aaatctgtga tgagctgatt gcaaagctgg gaaagactga ctgagacact ccccctgtta
1381 gctcaacaga tctgcatttg gctgcttctc ttgtgaccac aattatcttg ccttatccag
1441 gaataattgc ccctttgcag agaaaaaaaa aaacttaaaa aaagcacatg cctactgctg
1501 cctgtcccgc tttgctgcca atgcaacagc cctggaagaa accctagagg gttgcatagt
1561 ctagaaagga gtgtgacctg acagtgctgg agcctcctag tttcccccta tgaaggttcc
1621 cttaggctgc tgagtttggg tttgtgattt atctttagtt tgttttaaag tcatctttac
1681 tttcccaaat gtgttaaatt tgtaactcct ctttggggtc ttctccacca cctgtctgat
1741 ttttttgtga tctgtttaat cttttaattt tttagtatca gtggttttat ttaaggagac
1801 agtttggcct attgttactt ccaatttata atcaagaagg ggctctggat ccccttttaa
1861 attacacaca ctctcacaca catacatgta tgtttataga tgctgctgct cttttccctg
1921 aagcatagtc aagtaagaac tgctctacag aaggacatat ttccttggat gtgagaccct
1981 attttgaaat agagtcctga ctcagaacac caacttaaga atttgggggа ttaaagatgt
2041 gaagaccaca gtcttgggtt ttcatatctg gagaagacta tttgccatga cgttttgttg
2101 ccctggtatt tggacactcc tcagctttaa tgggtgtggc ccctttaggg ttagtcctca
2161 gactaatgat agtgtctgct ttctgcatga acggcaatat gggactccct ccaagctagg
2221 gtttggcaag tctgccctag agtcatttac tctcctctgc ctccatttgt taatacagaa
2281 tcaacattta gtcttcatta tctttttttt ttttttgag acagagtttc gatctatttt
2341 aagtatgtga agaaaatcta cttgtaaaag gctcagatct taattaaaag gtaattgtag
2401 cacattacca attataaggt gaagaaatgt ttttttccca agtgtgatgc attgttcttc
2461 agatgttgaa aagaaagcaa aaaatacctt ctaacttaag acagaatttt taacaaaatg
2521 agcagtaaaa gtcacatgaa ccactccaaa aatcagtgca ttttgcatat ttttaaacaa
2581 agacagcttg ttgaatactg agaagaggag tgcaaggaga aggtctgtac taacaaagcc
2641 aaattcctca agctcttact ggactcagtt cagagtggtg ggccattaac cccaacatgg
2701 aatttttcca tataaatctc aatgaattcc ctttcatttg aataggcaaa cccaaatcca
2761 tgcaagtgtt ttaaagcact gtcctgtctt aatcttacat gctgaaagtc ttcatggtga
2821 tatgcactat attcagtata cgtatgtttt cctacttctc ttgtaaaact gttgcatgat
2881 ccaacttcag caatgaattg tgcctagtgg agaacctcta tagatcttaa aaaatgaatt
2941 attctttagc agtgtattac tcacatgggt gcaatcttta gccccaggga ggtcaataat
3001 gtcttttaaa gccagaagtc acattttacc aatatgcatt tatcataatt ggtgcttagg
3061 ctgtatattc aagcctgttg tcttaacatt ttgtataaaa aagaacaaca gaaattatct
3121 gtcatttgag aagtggcttg acaatcattt gagctttgaa agcagtcact gtggtgtaat
3181 atgaatgctg tcctagtggt catagtacca agggcacgtg tctccccttg gtataactga
3241 tttccttttt agtcctctac tgctaaataa gttaattttg cattttgcag aaagaaacat
3301 tgattgctaa atctttttgc tgctgtgttt tggtgttttc atgtttactt gttttatatt
3361 gatctgtttt aagtatgaga ggcttatagt gccctccatt gtaaatccat agtcatcttt
3421 ttaagcttat tgtgtttaag aaagtagcta tgtgttaaac agaggtgatg gcagcccttc
3481 cctagcacac tggtggaaga gacccccttaa gaacctgacc ccagtgaatg aagctgatgc
3541 acagggagca ccaaaggacc ttcgttaagt gataattgtc ctggcctctc agccatgacc
3601 gttatgagga aatatccccc attcgaactt aacagatgcc tcctctccaa agagaattaa
3661 aatcgtagct tgtacagatc aagagaatat actgggcaga atgaagtatg tttgtttatt
3721 tttctttaaa aataaaggat tttggaactc tggagagtaa gaatatagta tagagtttgc
3781 ctcaacacat gtgagggcca aataacctgc tagctaggca gtaataaact ctgttacaga
3841 agagaaaaag ggccgggcac agtggcttat tcctgtaatc ccaacactgt ggaaggccga
3901 ggcaggagga tcacttgagt ccaggagttt gaaacctacc taggcaacat ggtgaaacct
3961 tgtctctacc aaaataaaaa ttagctgggc atggtggcac gtgcctgtgg tcccagctac
4021 ttgggaggct gaggtgggag cctgggaggt caaggctgca gtgagccatg atcatgccac
4081 tgcactccat cctgggtgac agcaagatct tgtctc
```

Figure 10 (cont.)

SEQ ID NO:29

```
   1 cgagttcccc gaggtgtacg tgcccaccgt cttcgagaac tatgtggccg acattgaggt
  61 ggacggcaag caggtggagc tggcgctgtg ggacacggcg ggccaggagg actacgaccg
 121 cctgcggccg ctctcctacc cggacaccga cgtcattctc atgtgcttct cggtggacag
 181 cccggactcg ctggagaaca tccccgagaa gtgggtcccc gaggtgaagc acttctgtcc
 241 caatgtgccc atcatcctgg tggccaacaa aaaagacctg cgcagcgacg agcatgtccg
 301 cacagagctg gcccgcatga agcaggaacc cgtgcgcacg gatgacggcc gcgccatggc
 361 cgtgcgcatc caagcctacg actacctcga gtgctctgcc aagaccaagg aaggcgtgcg
 421 cgaggtcttc gagacggcca cgcgcgccgc gctgcagaag cgctacggct cccagaacgg
 481 ctgcatcaac tgctgcaagg tgctatgagg gccgcgcccg tcgcgcctgc ccctgccggc
```

SEQ ID NO:30

```
   1 cggggagacc atggggcccc tctcagcccc ttcctgcaca cacctcatca cttggaaggg
  61 ggtcctgctc acagcatcac ttttaaactt ctggaatccg cccaccactg ccgaagtcac
 121 gattgaagcc cagccaccca aagtttctga ggggaaggat gttcttctac ttgttcacaa
 181 tttgccccag aatcttcctg gctacttctg gtacaaaggg gaaatgacgg acctctacca
 241 ttacattata tcgtatatag ttgatggtaa aataattata tatgggcctg catacagtgg
 301 aagagaaaca gtatattcca acgcatccct gctgatccag aatgtcaccc ggaaggatgc
 361 aggaacctac accttacaca tcataaagcg aggtgatgag actagagaag aaattcgaca
 421 tttcaccttc accttatact atggtccaga cctccccaga atttaccctt cattcaccta
 481 ttacggttca ggagaaaacc tcgacttgtc ctgcttcacg gaatctaacc caccggcaga
 541 gtatttttgg acaattaatg ggaagtttca gcaatcagga caaaagctct ttatccccca
 601 aattactaga aatcatagcg ggctctatgt ttgctctgtt cataactcag ccactggcaa
 661 ggaaatctcc aaatccatga cagtcaaagt ctctggtccc tgccatggag acctgacaga
 721 gtttcagtca tgactgcaac aactgagaca ctgagaaaaa gaacaggctg ataccttcat
 781 gaaattcaag acaaagaaga aaaaaactca atgttattgg actaaataat caaaaggata
 841 atgttttcat aatttttttat tggaaaatgt gctgattctt tgaatgtttt attctccaga
 901 tttatgaact ttttttcttc agcaattggt aaagtatact tttgtaaaca aaaattgaaa
 961 tatttgcttt tgctgtctat ctgaatgccc cagaattgtg aaactactca tgagtactca
1021 taggtttatg gtaataaagt tatttgcaca tgttccgtag ttt
```

SEQ ID NO:31

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   1 gcaccaacca gcaccatgcc catgatactg gggtactggg acatccgcgg gctggcccac
  61 gccatccgcc tgctcctgga atacacagac tcaagctatg aggaaaagaa gtacacgatg
 121 ggggacgctc ctgattatga cagaagccag tggctgaatg aaaaattcaa gctgggcctg
 181 gactttccca atctgcccta cttgattgat ggggctcaca agatcaccca gagcaacgcc
 241 atcttgtgct acattgcccg caagcacaac ctgtgtgggg agacagaaga ggagaagatt
 301 cgtgtggaca ttttggagaa ccagaccatg gacaaccata tgcagctggg catgatctgc
 361 tacaatccag aatttgagaa actgaagcca aagtacttgg aggaactccc tgaaaagcta
 421 aagctctact cagagtttct ggggaagcgg ccatggtttg caggaaacaa gatcactttt
 481 gtagattttc tcgtctatga tgtccttgac ctccaccgta tatttgagcc caactgcttg
 541 gacgccttcc caaatctgaa ggacttcatc tcccgctttg agggcttgga gaagatctct
 601 gcctacatga agtccagccg cttcctccca agacctgtgt tctcaaagat ggctgtctgg
 661 ggcaacaagt agggccttga aggcaggagg tgggagtgag gagcccatac tcagcctgct
 721 gcccaggctg tgcagcgcag ctggactctg catcccagca cctgcctcct cgttcctttc
 781 tcctgtttat tcccatcttt actcccaaga cttcattgtc cctcttcact ccccctaaac
 841 ccctgtccca tgcaggccct ttgaagcctc agctacccac tatccttcgt gaacatcccc
 901 tcccatcatt acccttccct gcactaaagc cagcctgacc ttccttcctg ttagtggttg
 961 tgtctgcttt aaagcctgcc tggcccctcg cctgtggagc tcagccccga gctgtccccg
1021 tgttgcatga aggagcagca ttgactggtt tacaggccct gctcctgcag catggtccct
1081 gcctaggcct acctgatgga agtaaagcct caaccac
```

SEQ ID NO:32

```
   1 ttcaggaacc ggtttggtgc tggtgctgga ggcggctatg gctttggagg tggtgccggt
```

Figure 10 (cont.)

```
  61 agtggatttg gtttcggcgg tggagctggt ggtggctttg ggctcggtgg cggagctggc
 121 tttggaggtg gcttcggtgg ccctggcttt cctgtctgcc ctcctggagg tatccaagag
 181 gtcactgtca accagagtct cctgactccc ctcaacctgc aaatcgaccc cagcatccag
 241 agggtgagga ccgaggagcg cgagcagatc aagaccctca acaataagtt tgcctccttc
 301 atcgacaagg tgcggttcct ggagcagcag aacaaggttc tggacaccaa gtggaccctg
 361 ctgcaggagc agggcaccaa gactgtgagg cagaacctgg agccgttgtt cgagcagtac
 421 atcaacaacc tcaggaggca gctggacagc atcgtggggg aacggggccg cctggactca
 481 gagctgagaa acatgcagga cctggtggaa gacttcaaga acaagtatga ggatgaaatc
 541 aacaagcgta ccactgctga gaatgagttt gtgatgctga agaaggatgt agatgctgcc
 601 tacatgaaca aggtggagct ggaggccaag gttgatgcac tgatggatga gattaacttc
 661 atgaagatgt tctttgatgc ggagctgtcc cagatgcaga cgcatgtctc tgacacctca
 721 gtggtcctct ccatggacaa caaccgcaac ctggacctgg atagcatcat cgctgaggtc
 781 aaggcccagt atgaggagat tgccaaccgc agccggacag aagccgagtc ctggtatcag
 841 accaagtatg aggagctgca gcagacagct ggccggcatg gcgatgacct ccgcaacacc
 901 aagcatgaga tctctgagat gaaccggatg atccagaggc tgagagccga gattgacaat
 961 gtcaagaaac agtgcgccaa tctgcagaac gccattgcgg atgccgagca gcgtggggag
1021 ctggccctca aggatgccag gaacaagctg gccgagctgg aggaggccct gcagaaggcc
1081 aagcaggaca tggcccggct gctgcgtgag taccaggagc tcatgaacac caagctggcc
1141 ctggacgtgg agatcgccac ttaccgcaag ctgctggagg gcgaggaatg cagactcagt
1201 ggagaaggag ttggaccagt caacatctct gttgtcacaa gcagtgtttc ctctggatat
1261 ggcagtggca gtggctatgg cggtggcctc ggtggaggtc ttggcggcgg cctcggtgga
1321 ggtcttgccg gaggtagcag tggaagctac tactccagca gcagtggggg tgtcggccta
1381 ggtggtgggc tcagtgtggg gggctctggc ttcagtgcaa gcagtggccg agggctgggg
1441 gtgggctttg gcagtggcgg gggtagcagc tccagcgtca aatttgtctc caccacctcc
1501 tcctcccgga agagcttcaa gagctaagaa cctgctgcaa gtcactgcct tccaagtgca
1561 gcaacccagc ccatggagat tgcctcttct aggcagttgc tcaagccatg ttttatcctt
1621 ttctggagag tagtctagac caagccaatt gcagaaccac attctttggt tcccaggaga
1681 gccccattcc cagcccctgg tctcccgtgc cgcagttcta tattctgctt caaatcagcc
1741 ttcaggtttc ccacagcatg gcccctgctg acacgagaac ccaaagtttt cccaaatcta
1801 aatcatcaaa acagaatccc caccccaatc ccaaattttg ttttggttct aactacctcc
1861 agaatgtgt
```

SEQ ID NO:33

```
   1 agtgatcagg gccaaagcgg tcagtgagaa ggaagtggac tctggaaacg acatttatgg
  61 caaccctatc aagaggatcc agtatgagat caagcagata aagatgttca aagggcctga
 121 gaaggatata gagtttatct acacggcccc ctcctcggca gtgtgtgggg tctcgctgga
 181 cgttggagga aagaaggaat atctcattgc aggaaaggcc gaggggacg gcaagatgca
 241 catcaccctc tgtgacttca tcgtgccctg ggacaccctg agcaccaccc agaagaagag
 301 cctgaaccac aggtaccaga tgggctgcga gtgcaagatc acgcgctgcc ccatgatccc
 361 gtgctacatc tcctccccgg acgagtgcct ctggatggac tgggtcacag agaagaacat
 421 caacgggcac caggccaagt tcttcgcctg catcaagaga agtgacggct cctgtgcgtg
 481 gtaccgcggc gcggcgcccc ccaagcagga gtttctcgac atcgaggacc cataagcagg
 541 cctccaacgc ccctgtggcc aactgcaaaa aaagcctcca agggtttcga ctggtccagc
 601 tctgacatcc cttcctggaa acagcatgaa taaaacactc atcccatggg tccaaattaa
 661 tatg
```

SEQ ID NO:34

```
   1 tgtcgccacc atggctccgc accgccccgc gcccgcgctg ctttgcgcgc tgtccctggc
  61 gctgtgcgcg ctgtcgctgc ccgtccgcgc ggccactgcg tcgcgggggg cgtcccaggc
 121 gggggcgccc cagggggcgg tgcccgaggc gcggcccaac agcatggtgg tggaacaccc
 181 cgagttcctc aaggcaggga aggagcctgg cctgcagatc tggcgtgtgg agaagttcga
 241 tctggtgccc gtgcccacca acctttatgg agacttcttc acgggcgacg cctacgtcat
 301 cctgaagaca gtgcagctga ggaacggaaa tctgcagtat gacctccact actggctggg
 361 caatgagtgc agccaggatg agagcggggc ggccgccatc tttaccgtgc agctggatga
```

Figure 10 (cont.)

```
 421 ctacctgaac ggccgggccg tgcagcaccg tgaggtccag ggcttcgagt cggccacctt
 481 cctaggctac ttcaagtctg gcctgaagta caagaaagga ggtgtggcat caggattcaa
 541 gcacgtggta cccaacgagg tggtggtgca gagactcttc caggtcaaag ggcggcgtgt
 601 ggtccgtgcc accgaggtac ctgtgtcctg ggagagcttc aacaatggcg actgcttcat
 661 cctggacctg ggcaacaaca tccaccagtg gtgtggttcc aacagcaatc ggtatgaaag
 721 actgaaggcc acacaggtgt ccaagggcat ccgggacaac gagcggagtg gccgggcccg
 781 agtgcacgtg tctgaggagg gcactgagcc cgaggcgatg ctccaggtgc tgggccccaa
 841 gccggctctg cctgcaggta ccgaggacac cgccaaggag gatgcggcca accgcaagct
 901 ggccaagctc tacaaggtct ccaatggtgc agggaccatg tccgtctccc tcgtggctga
 961 tgagaacccc ttcgcccagg gggccctgaa gtcagaggac tgcttcatcc tggaccacgg
1021 caaagatggg aaaatctttg tctggaaagg caagcaggca aacacggagg agaggaaggc
1081 tgccctcaaa acagcctctg acttcatcac caagatggac taccccaagc agactcaggt
1141 ctcggtcctt cctgagggcg gtgagacccc actgttcaag cagttcttca agaactggcg
1201 ggacccagac cagacagatg gcctgggctt gtcctacctt tccagccata tcgccaacgt
1261 ggagcgggtg cccttcgacg ccgccaccct gcacacctcc actgccatgg ccgcccagca
1321 cggcatggat gacgatggca caggccagaa acagatctgg agaatcgaag gttccaacaa
1381 ggtgcccgtg gaccctgcca catatggaca gttctatgga ggcgacagct acatcattct
1441 gtacaactac cgccatggtg gccgccaggg gcagataatc tataactggc agggtgccca
1501 gtctacccag gatgaggtcg ctgcatctgc catcctgact gctcagctgg atgaggagct
1561 gggaggtacc cctgtccaga gccgtgtggt ccaaggcaag gagcccgccc acctcatgag
1621 cctgtttggt gggaagccca tgatcatcta caagggcggc acctcccgcg agggcgggca
1681 gacagcccct gccagcaccc gcctcttcca ggtccgcgcc aacagcgctg gagccacccg
1741 ggctgttgag gtattgccta aggctggtgc actgaactcc aacgatgcct ttgttctgaa
1801 aacccctca gccgcctacc tgtgggtggg tacaggagcc agcgaggcag agaagacggg
1861 ggcccaggag ctgctcaggg tgctgcgggc ccaacctgtg caggtggcag aaggcagcga
1921 gccagatggc ttctgggagg ccctgggcgg gaaggctgcc taccgcacat ccccacggct
1981 gaaggacaag aagatggatg cccatcctcc tcgcctcttt gcctgctcca acaagattgg
2041 acgttttgtg atcgaagagg ttcctggtga gctcatgcag gaagacctgg caacggatga
2101 cgtcatgctt ctggacacct gggaccaggt ctttgtctgg gttggaaagg attctcaaga
2161 agaagaaaag acagaagcct tgacttctgc taagcggtac atcgagacgg acccagccaa
2221 tcgggatcgg cggacgccca tcaccgtggt gaagcaaggc tttgagcctc cctcctttgt
2281 gggctggttc cttggctggg atgatgatta ctggtctgtg gaccccttgg acagggccat
2341 ggctgagctg gctgcctgag gaggggcagg gcccacccat gtcaccggtc agtgcctttt
2401 ggaactgtcc ttccctcaaa gaggccttag agcgagcaga gcagctctgc tatgagtgtg
2461 tgtgtgtgtg tgtgttgttt ctttttttt tttttacagt atccaaaaat agccctgcaa
2521 aaattcagag tccttgcaaa attgtctaaa atgtcagtgt ttgggaaatt aaatccaata
2581 aaaacatttt gaagtgtg
```

SEQ ID NO:35

```
gaagtaaaagatttttattgttctatagacacttctgaaaagagatctaattgagaaaat
atacaaagcatttaagagtttcatccccagagactgactgaaggcgttacagccctcctc
tccaaggctcagggctgagaacggttagcatatcgaatgatcagtaaaaacatgcaaaag
tgagaaggaaagggaaaaaggtgcattcccctaagctgaggggatggaatttcagaaca
gaggangcagggtggacaagtaccaaggtggctctccctttccctctgtgtnatctttca
aaaccanttccaagcntggatnaaagcaa
```

SEQ ID NO:36

```
   1 caaagtctga gccccgctcc gctgatgcct gtctgcagaa tccgcaccaa ccagcaccat
  61 gcccatgact ctggggtact gggacatccg tgggctggcc cacgccatcc gcttgctcct
 121 ggaatacaca gactcaagct atgtggaaaa gaagtacacg ctgggggacg ctcctgacta
 181 tgacagaagc cagtggctga atgaaaaatt caagctgggc ctggactttc ccaatctgcc
 241 ctacttgatt gatggggctc acaagatcac ccagagcaat gccatcctgc gctacattgc
 301 ccgcaagcac aacctgtgtg gggagacaga agaggagaag attcgtgtgg acattttgga
 361 gaaccaggtt atggataacc acatggagct ggtcagactg tgctatgacc cagattttga
```

Figure 10 (cont.)

```
 421 gaaactgaag ccaaaatact tggaggaact ccctgaaaag ctaaagctct actcagagtt
 481 tctggggaag cggccatggt ttgcaggaga caagatcacc tttgtggatt tccttgccta
 541 tgatgtcctt gacatgaagc gtatatttga gcccaagtgc ttggacgcct tcctaaactt
 601 gaaggacttc atctcccgct ttgagggttt gaagaagatc tctgcctaca tgaagtccag
 661 ccaattcctc cgaggtcttt tgtttggaaa gtcagctaca tggaacagca aatagggccc
 721 agtgatgcca gaagatggga gggaggagcc aaccttgctg cctgcgaccc tggaggacag
 781 cctgactccc tggacctgcc ttcttccttt ttccttcttt ctactctctt ctcttcccca
 841 aggcctcatt ggcttccttt cttctaacat catccctccc cgcatcgagg ctctttaaag
 901 cttcagctcc ccactgtcct ccatcaaagt ccccctccta acgtcttcct ttccctgcac
 961 taacgccaac ctgactgctt ttcctgtcag tgcttttctc ttctttgaga agccagactg
1021 atctctgagc tcccctagcac tgtcctcaaa gaccatctgt atgccctgct ccctttgctg
1081 ggtccctacc ccagctccgt gtgatgccca gtaaagcctg aaccatgcct gccatgtctt
1141 gtcttattcc ctgaggctcc cttgactcag gactgtgctc gaattgtggg tggtttttg
1201 tcttctgttg tccacagcca gagcttagtg gatgggtgtg tgtgtgtgtg tgttgggggt
1261 ggtgatcagg caggttcata aatttccttg gtcatttctg ccctctagcc acatccctct
1321 gttcctcact gtggggatta ctacagaaag gtgctctgtg ccaagttcct cactcattcg
1381 cgctcctgta ggccgtctag aactggcatg gttcaaagag gggctaggct gatggggaag
1441 ggggctgagc agctcccagg cagactgcct tctttcaccc tgtcctgata gacttccctg
1501 atctagatat ccttcgtcat gacacttctc aataaaacgt atcccaccgt attgt
```

SEQ ID NO:37

```
   1 ggttgagaat gcttgcacca agcttgtcca ggcagctcag atgcttcagt cagacccttа
  61 ctcagtgcct gctcgagatt atctaattga tgggtcaagg ggcatcctct ctggaacatc
 121 agacctgctc cttaccttcg atgaggctga ggtccgtaaa attattagag tttgcaaagg
 181 aattttggaa tatcttacag tggcagaggt ggtggagact atggaagatt tggtcactta
 241 cacaaagaat cttgggccag gaatgactaa gatggccaag atgattgacg agagacagca
 301 ggagctcact caccaggagc accgagtgat gttggtgaac tcgatgaaca ccgtgaaaga
 361 gttgctgcca gttctcattt cagctatgaa gatttttgta acaactaaaa actcaaaaaa
 421 ccaaggcata gaggaagctt taaaaaatcg caattttact gtagaaaaaa tgagtgctga
 481 aattaatgag ataattcgtg tgttacaact cacctcttgg gatgaagatg cctgggccag
 541 caaggacact gaagccatga agagagcatt ggcctccata gactccaaac tgaaccaggc
 601 caaaggttgg ctccgtgacc ctagtgcctc cccaggggat gctggtgagc aggccatcag
 661 acagatctta gatgaagctg gaaaagttgg tgaactctgt gcaggcaaag aacgcaggga
 721 gattctggga acttgcaaaa tgctagggca gatgactgat caagtggctg acctccgtgc
 781 cagnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
 841 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
 901 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
 961 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
1021 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
1081 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnaggctcg
1141 agccttggcc aaacaggtgg ccacggccct gcagaacctg cagaccaaaa ccaaccgggc
1201 tgtggccaac agcagaccgg ccaaagcagc tgtacacctt gagggcaaga ttgagcaagc
1261 acagcggtgg attgataatc ccacagtgga tgaccgtgga gtcggtcagg ctgccatccg
1321 ggggcttgtg gccgaagggc atcgtctggc taatgttatg atggggcctt atcggcaaga
1381 tcttctcgcc aagtgtgacc gagtggacca gctgacagcc cagctggctg acctggctgc
1441 cagaggggaa ggggagagtc ctcaggcacg agcacttgca tctcagctcc aagactcctt
1501 aaaggatcta aaagctcgga tgcaggaggc catgactcag gaagtgtcag atgttttcag
1561 cgataccaca actcccatca agctgttggc agtggcagcc acggcgcctc ctgatgcgcc
1621 taacagggaa gaggtatttg atgagagggc agctaacttt gaaaaccatt caggaaagct
1681 tggtgctacg gccgagaagg cggctgcggt tggtactgct aataaatcaa cagtggaagg
1741 cattcaggcc tcagtgaaga cggcccgaga actcacaccc caggtggtct cggctgctcg
1801 tatcttactt aggaaccctg gaaatcaagc tgcttatgaa cattttgaga ccatgaagaa
1861 ccagtggatc gataatgttg aaaaaatgac agggctggtg gacgaagcca ttgataccaa
```

Figure 10 (cont.)

```
1921 atctctgttg gatgcttcag aagaagcaat taaaaaagac ctggacaagt gcaaggtagc
1981 tatggccaac attcagcctc agatgctggt tgctggggca accagtattg ctcgtcgggc
2041 caaccggatc ctgctggtgg ctaagaggga ggtggagaat tccgaggatc ccaagttccg
2101 tgaggctgtg aaagctgcct ctgatgaatt gagcaaaacc atctccccga tggtgatgga
2161 tgcaaaagct gtggctggaa acatttccga ccctggactg caaaagagct tcctggactc
2221 aggatatcgg atcctgggag ctgtggccaa ggtcagagaa gccttccaac ctcaggagcc
2281 tgacttcccg ccgcctccac cagaccttga acaactccga ctaacagatg agcttgctcc
2341 tcccaaacca cctctgcctg aaggtgaggt ccctccacct aggcctccac caccagagga
2401 aaaggatgaa gagttccctg agcagaaggc cggggaggtg attaaccagc caatgatgat
2461 ggctgccaga cagctccatg atgaagctcg caaatggtcc agcaagggca atgacatcat
2521 tgcagcagcc aagcgcatgg ctctgctgat ggctgagatg tctcggctgg taagaggggg
2581 cagtggtacc aagcgggcac tcattcagtg tgccaaggac atcgccaagg cctcagatga
2641 ggtgactcgg ttggccaagg aggttgccaa gcagtgcaca gataaacgga ttagaaccaa
2701 cctcttacag gtatgtgagc gaatcccaac cataagcacc cagctcaaaa tcctgtccac
2761 agtgaaggcc accatgctgg gccggaccaa catcagtgat gaggagtctg agcaggccac
2821 agagatgctg gttcacaatg cccagaacct catgcagtct gtgaaggaga ctgtgcggga
2881 agctgaagct gcttcaatca aaattcgaac agatgctgga tttacactgc gctgggttag
2941 aaagactccc tggtaccagt aggcacctgg ctgagcctgg ctggcacaga aacctctact
3001 aaaaagaagg aaaatgatct gagtcccagg agctgcccag agttgctggg agctgaaaaa
3061 tcacatcctg gcctggcaca tcagaaagga atgggggcct cttcaaatta gaagacattt
3121 atactctttt ttcatggaca ctttgaaatg tgtttctgta taaagcctgt attctcaaac
3181 acagttacac ttgtgcaccc tctatcccaa taggcagact gggtttctag cccatggact
3241 tcacataagc tcagaatcca agtgaacact agccagacac tctgctctgc ccttgttccc
3301 taggggacac ttccctctgt ttctctttcc ttggctccca ttcactcttc cagaatccca
3361 agacccaggg cccaggcaaa tcagttacta agaagaaaat tgctgtgcct cccaaaattg
3421 ttttgagctt tccatgttgc tgccaaccat accttccttc cctgggctgt gctacctggg
3481 tccttttcag aagtgagctt tgctgctaca ggggaaggtg gcctctgtgg agccccagca
3541 tatgggggcc tggattcatt tcctgccctt cctcagttta atccttctag tttcccacaa
3601 tataaaactg tacttcactg tcaggaagaa atcacagaat catatgattc tgcttttacc
3661 atgcccctga gcaatgtctg tgctagggaa acttcccgtc ccatatcctg cctcagcccg
3721 ccaaggtagc catcccatga acacactgtg tcctggtgct ctctgccact ggaagggcag
3781 agtagccagg gtgtggccct gccatcttcc cagcagggcc actcccggca ctccatgctt
3841 agtcactgcc tgcagaggtc tgtgctgagg ccttatcatt cattcttagc tcttaattgt
3901 tcattttgag ctgaaatgct gcattttaat tttaaccaaa acatgtctcc tatcctggtt
3961 tttgtagcct tcctccacat cctttctaaa caagatttta aagacatgta ggtgtttgtt
4021 catctgtaac tctaaaagat ccttttttaaa ttcagtccta agaaagagga gtgcttgtcc
4081 cctaagagtg tttaatggca aggcagccct gtctgaagga cacttcctgc ctaagggaga
4141 gtggtatttg cagactagaa ttctagtgct gctgaagatg aatcaatggg aaatactact
4201 cctgtaattc ctacctccct gcaaccaact acaaccaagc tctctgcatc tactcccaag
4261 tatggggttc aagagagtaa tgggtttcat atttcttatc accacagtaa gttcctacta
4321 ggcaaaatga gagggcagtg tttccttttt ggtacttatt actgctaagt atttcccagc
4381 acatgaaacc ttatttttc ccaaagccag aaccagatga gtaaaggagt aagaaccttg
4441 cctgaacatc cttccttccc acccatcgct gtgtgttagt tcccaacatc gaatgtgtac
4501 aacttaagtt ggtcctttac actcaggctt tcactatttc ctttataatg aggatgatta
4561 ttttcaaggc cctcagcata tttgtatagt tgcttgcctg atataaatgc aatattaatg
4621 cctttaaagt atgaatctat gccaaagatc acttgttgtt ttactaaaga aagattactt
4681 agaggaaata agaaaaatca tgtttgctct cccggttctt ccagtggttt gagacactgg
4741 tttacacttt atgccggatg tgcttttctc caatatcagt gctcgagaca cagtgaagca
4801 aattaaaaaa aa
```

SEQ ID NO:38

```
   1 atatccagcc tttgccgaat acatcctatc tgccacacat ccagcgtgag gtccctccag
  61 ctacaaggtg ggcaccatgg cggagaagtt tgactgccac tactgcaggg atcccttgca
 121 ggggaagaag tatgtgcaaa aggatggcca ccactgctgc ctgaaatgct ttgacaagtt
```

Figure 10 (cont.)

```
 181 ctgtgccaac acctgtgtgg aatgccgcaa gcccatcggt gcggactcca aggaggtgca
 241 ctataagaac cgcttctggc atgacacctg cttccgctgt gccaagtgcc ttcacccctt
 301 ggccaatgag acctttgtgg ccaaggacaa caagatcctg tgcaacaagt gcaccactcg
 361 ggaggactcc cccaagtgca aggggtgctt caaggccatt gtggcaggag atcaaaacgt
 421 ggagtacaag gggaccgtct ggcacaaaga ctgcttcacc tgtagtaact gcaagcaagt
 481 catcgggact ggaagcttct tccctaaagg ggaggacttc tactgcgtga cttgccatga
 541 gaccaagttt gccaagcatt gcgtgaagtg caacaaggcc atcacatctg gaggaatcac
 601 ttaccaggat cagccctggc atgccgattg ctttgtgtgt gttacctgct ctaagaagct
 661 ggctgggcag cgtttcaccg ctgtggagga ccagtattac tgcgtggatt gctacaagaa
 721 ctttgtggcc aagaagtgtg ctggatgcaa gaaccccatc actgggaaaa ggactgtgtc
 781 aagagtgagc cacccagtct ctaaagctag gaagccccca gtgtgccacg ggaaacgctt
 841 gcctctcacc ctgtttccca gcgccaacct ccggggcagg catccgggtg gagagaggac
 901 ttgtccctcg tgggtggtgg ttctttatag aaaaaatcga agcttagcag ctcctcgagg
 961 cccgggtttg gtaaaggctc cagtgtggtg gcctatgaag gacaatcctg gcacgactac
1021 tgcttccact gcaaaaaatg ctccgtgaat ctggccaaca agcgctttgt tttccaccag
1081 gagcaagtgt attgtcccga ctgtgccaaa aagctgtaaa ctgacagggg ctcctgtcct
1141 gtaaaatggc atttgaatct cgttctttgt gtccttactt tctgccctat accatcaata
1201 ggggaagagt ggtccttccc ttctttaaag ttctccttcc gtctttttctc ccattttaca
1261 gtattactca aataagggca cacagtgatc atattagcat ttagcaaaaa gcaaccctgc
1321 agcaaagtga atttctgtcc ggctgcaatt taaaaatgaa aacttaggta gattgactct
1381 tctgcatgtt tctcatagag cagaaaagtg ctaatcattt agccacttag tgatgtaagc
1441 aagaagcata ggagataaaa cccccactga gatgcctctc atgcctcagc tgggacccac
1501 cgtgtagaca cacgacatgc aagagttgca gcggctgctc caactcactg ctcaccctct
1561 tctgtgagca ggaaaagaac cctactgaca tgcatggttt aacttcctca tcagaactct
1621 gcccttcctt ctgttctttt gtgctttcaa ataactaaca cgaacttcca gaaaattaac
1681 atttgaactt agctgtaatt ctaaactgac ctttccccgt actaacgttt ggtttccccg
1741 tgtggcatgt tttctgagcg ttcctacttt aaagcatgga acatgcaggt gatttgggaa
1801 gtgtagaaag acctgagaaa acgagcctgt ttcagaggaa catcgtcaca acgaatactt
1861 ctggaagctt aacaaaacta accctgctgt ccttttttatt gtttttaatt aatatttttg
1921 ttttaattga tagcaaaata gtttatgggt ttggaaactt gcatgaaaat attttagccc
1981 cctcagatgt tcctgcagtg ctgaaattca tcctacggaa gtaaccgcaa aactctag
```

SEQ ID NO:39

```
   1 tgccgcccta caccgtggtc tatttcccag ttcgagnnnn nnnnnnnnnn nnnnnnnnnn
  61 nnnnnnnnnn nnnnnnnngc tgctggcaga tcagggccag agctggaagg aggaggtggt
 121 gaccgtggag acgtggcagg agggctcact caaagcctcc tgcctatacg ggcagctccc
 181 caagttccag gacggagacc tcaccctgta ccagtccaat accatcctgc gtcacctggn
 241 nnnnnnnnnn nnnnnnnnnn nnnnnnnngg ctctatggga aggaccagca ggaggcagcc
 301 ctggtggaca tggtgaatga cggcgtggag gacctccgct gcaaatacat ctccctcatc
 361 tacaccaact atgaggcggg caaggatgac tatgtgaagg cactgcccgg gcaactgaag
 421 ccttttgaga ccctgctgtc ccagaaccag ggaggcaaga ccttcattgt gggagaccag
 481 atctccttcg ctgactacaa cctgctggac ttgctgctga tccatgaggt cctagcccct
 541 ggctgcctgg atgcgttccc cctgctctca gcatatgtgg ggcgcctcag tgcccggccc
 601 aagctcaagg ccttcctggc ctcccctgag tacgtgaacc tccccatcaa tggcaacggg
 661 aaacagtgag ggttgggggg actctgagcg g
```

SEQ ID NO:40

```
   1 cttttcacac tggccttaaa gaggatatat tagaagttga agtaggaagg gagccagaga
  61 ggccgatggc gcaaaggtac gacgatctac cccattacgg gggcatggat ggagtaggca
 121 tcccctccac gatgtatggg gacccgcatg cagccaggtc catgcagccg gtccaccacc
 181 tgaaccacgg gcctcctctg cactcgcatc agtacccgca cacagctcat accaacgcca
 241 tggcccccag catgggctcc tctgtcaatg acgctttaaa gagagataaa gatgccattt
 301 atggacaccc cctcttccct ctcttagcac tgatttttga gaaatgtgaa ttagctactt
```

Figure 10 (cont.)

```
 361 gtacccccg cgagccgggg gtggcgggcg gggacgtctg ctcgtcagag tcattcaatg
 421 aagatatagc cgtgttcgcc aaacagattc gcgcagaaaa acctctattt tcttctaatc
 481 cagaactgga taacttgatg attcaagcca tacaagtatt aaggtttcat ctattggaat
 541 tagagaaggt acacgaatta tgtgacaatt tctgccaccg gtatattagc tgtttgaaag
 601 ggaaaatgcc tatcgatttg gtgatagacg atagagaagg aggatcaaaa tcagacagtg
 661 aagatataac aagatcagca aatctaactg accagccctc ttggaacaga gatcatgatg
 721 acacggcatc tactcgttca ggaggaaccc caggcccttc cagcggtggc cacacgtcac
 781 acagtgggga caacagcann nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
 841 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
 901 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnncaccctt
 961 acccttctga agaacagaaa aagcagttgg cacaagacac gggactcacc atccttcaag
1021 tgaacaattg gtttattaat gcccggagaa gaatagtgca gcccatgata gaccagtcca
1081 accgagcagt aagtcaagga acaccttata atcctgatgg acagcccatg ggaggtttcg
1141 taatggacgg tcagcaacat atgggaatta gagcaccagg acctatgagt ggaatgggca
1201 tgaatatggg catggagggg cagtggcact acatgtaacc ttcatctagt taaccaatcg
1261 caaagcaagg gggaaggctg caaagtatgc caggggagta tgtagcccgg ggtggtccaa
1321 tgggtgtgag tatgggacag ccaagttata cccaacccca gatgcccccc catcctgctc
1381 agctgcgtca tgggcccccc atgcatacgt acattcctgg acaccctcac cacccaacag
1441 tgatgatgca tggaggaccg ccccaccctg gaatgccaat gtcagcatca agcccacag
1501 ttcttaatac aggagaccca acaatgagtg gacaagtcat ggacattcat gctcagtagc
1561 ttaagggaat atgcattgtc tgcaatggtg actgatttca aatcatgttt tttctgcaat
1621 gactgtggag ttccattctt ggcatctact ctggaccaag gagcatccct aattcttcat
1681 agggaccttt aaaaagcagg aaataccaac tgaagtcaat ttgggggaca tgctaaataa
1741 ctatataaga cattaagaga acaaagagtg aaatattgta aatgctatta tactgttatc
1801 catattacgt tgtttcttat agatttttta aaaaaaatgt gaaatttttc cacactatgt
1861 gtgttgtttc catagctctt cacttcctcc agaagcctcc ttacattaaa aagccttaca
1921 gttatcctgc aagggacagg aaggtctgat ttgcaggatt tttagagcat taaaataact
1981 atcaggcaga agaatctttc ttctcgccta ggatttcagc catgcgcgcg ctctctctct
2041 ttctctctct tttcctctct ctccctcttt ctagcctggg gcttgaattt gcatgtctaa
2101 ttcatttact caccatattt gaattggcct gaacagatgt aaatcgggaa ggatgggaaa
2161 aactgcagtc atcaacaatg attaatcagc tgttgcaggc agtgtcttaa ggagactggt
2221 aggaggaggc atggaaacca aaaggccgtg tgtttagaag cctaattgtc acatcaagca
2281 tcattgtccc catgcaacaa ccaccacctt atacatcact tcctgtttta agcagctcta
2341 aaacatagac tgaagattta tttttaatat gttgacttta tttctgagca aagcatcggt
2401 catgtgtgta ttttttcata gtcccacctt ggagcattta tgtagacatt gtaaataaat
2461 tttgtgcaaa aaggactgga aaaatgaact gtattattgc aatttttttt t
```

SEQ ID NO:41

```
   1 ctcaataagc caaccatgtc tttcaaggat tacatccaag agaggagtga cccagtggag
  61 caaggcaaac cagttatacc tgcagctgtg ctggccggct tcacaggaag tggacctatt
 121 cagctgtggc agtttctcct ggagctgcta tcagacaaat cctgccagtc attcatcagc
 181 tggactggag acggatggga gtttaagctc gccgaccccg atgaggtggc ccgccggtgg
 241 ggaaagagga aaaataagcc caagatgaac tacgagaagc tgagccgggg cttacgctac
 301 tattacgaca agaacatcat ccacaagacg tcggggaagc gctacgtgta ccgcttcgtg
 361 tgcgacctcc agaacttgct ggggttcacg cccgaggaac tgcacgccat cctgggcgtc
 421 cagcccgaca cggaggactg a
```

SEQ ID NO:42

```
   1 ggacgacaag gcgttcacca aggagctgga ccagtgggtc gagcagctga acgagtgtaa
  61 gcagctgaac gagaaccaag tgcggacgct gtgcgagaag gcaaaggaaa ttttaacaaa
 121 agaatcaaat gtgcaagagg ttcgttgccc tgttactgtc tgtggagatg tgcatggtca
 181 atttcatgat cttatggaac tctttagaat tggtggaaaa tcaccggata caaactactt
 241 attcatgggt gactatgtag acagaggata ttattcagtg gagactgtga ctcttcttgt
 301 agcattaaag gtgcgttatc cagaacgcat tacaatattg agaggaaatc acgaaagccg
```

Figure 10 (cont.)

```
 361 acaaattacc caagtatatg gcttttatga tgaatgtctg cgaaagtatg ggaatgccaa
 421 cgtttggaaa tattttacag atctctttga ttatcttcca cttacagctt tagtagatgg
 481 acagatattc tgcctccatg gtggcctctc tccatccata gacacactgg atcatataag
 541 agccctggat cgtttacagg aagttccaca tgagggccca atgtgtgatc tgttatggtc
 601 agatccagat gatcgtggtg gatggggtat ttcaccacgt ggtgctggct acacatttgg
 661 acaagacatt tctgaaacct ttaaccatgc caatggtctc acactggttt ctcgtgccca
 721 ccagcttgta atggagggat acaattggtg tcatgatcgg aatgtggtta ccattttcag
 781 tgcacccaat tactgttatc gttgtgggaa ccaggctgct atcatggaat tagatgacac
 841 tttaaaatat tccttccttc aatttgaccc agcgcctcgt cgtggtgagc ctcatgttac
 901 acggcgcacc ccagactact tcctataaat ttctcctggg aaacctgcct ttgtatgtgg
 961 aagtatacct ggctttttaa aatatatgta tttaaaaaca aaaagcaaca gtaatctatg
1021 tgtttctgta acaaattggg atctgtcttg gcattaaacc acatcatgga ccaaatgtgc
1081 catactaatg atgagcattt agcacaattt gagactgaaa tttagtacac tatgttctag
1141 gtcagtctaa cagtttgcct gctgtattta tagtaaccat tttcctttgg actgttcaag
1201 caaaaaaggt aactaactgc ttcatctcct tttgcgctta tttggaaatt ttagttatag
1261 tgtttaactg gcatggatta atagagttgg agttttattt ttaagaaaaa ttcacaagct
1321 aacttccact aatccattat cctttatttt attgaaatgt ataattaact taactgaaga
1381 aaaggttctt cttgggagta tgttgtcata acatttaaag agatttccct tcatttaaac
1441 taaattactg ttttatgttg atctgcatat ttctgtatat ttgtcatgac agtgcttgca
1501 tcctatttgg tgtactcagc aaataaactt t
```

SEQ ID NO:43

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   1 cctgtgagca ccacgtcaac ggctcccggc ccccatgcac gggggaggga gataccccca
  61 agtgtagcaa gatctgtgag cctggctaca gcccgaccta caaacaggac aagcactacg
 121 gatacaattc ctacagcgtc tccaatagcg agaaggacat catggccgag atctacaaaa
 181 acggccccgt ggagggagct ttctctgtgt attcggactt cctgctctac aagtcaggag
 241 tgtaccaaca cgtcaccgga gagatgatgg gtggccatgc catccgcatc ctgggctggg
 301 gagtggagaa tggcacaccc tactggctgg ttgccaactc ctggaacact gactggggtg
 361 acaatggctt ctttaaaata ctcagaggac aggatcactg tggaatcgaa tcagaagtgg
 421 tggctggaat tccacgcacc gatcagtact gggaaaagat ctaatctgcc gtgggcctgt
 481 cgtgccagtc ctgggggcga gatgggggta gaaatgcatt ttattcttta agttcacgta
 541 agatacaagt ttcagacagg gtctgaagga ctggattggc caaacatcag acctgtcttc
 601 caaggagacc aagtcctggc tacatcccag cctgtggtta cagtgcagac aggccatgtg
 661 agccaccgct gccagcacag agcgtccttc cccctgtaga ctagtgccgt agggagtacc
 721 tgttgcccca gctgactgtg gccccctccg tgatccatcc atctccaggg agcaagacag
 781 agacccagga atggaaagcg gagttcctaa caggatgaaa gttcccccat cagttccccc
 841 agtacctcca agcaagtagc tttccacatt tgtcacagaa atcagaggag agatggtgtt
 901 gggagccctt tggagaacgc cagtctccca ggccccctgc atctatcgag tttgcaatgt
 961 cacaacctct ctgatcttgt gctcagcatg attctttaat agaagtttta ttttttcgtg
1021 cactctgcta atcatgtggg tgagccagtg gaacagcggg agacctgtgc tagttttaca
1081 gattgcctcc ttatgacgcg gctcaaaagg aaaccaagtg gtcaggagtt gtttctgacc
1141 cactgatctc tactaccaca aggagaatag tttaggagaa accagctttt actgtttttg
1201 aaaaattaca gcttcaccct gtcaagttaa caaggaatgc ctgtgccaat aaaaggtttc
```

SEQ ID NO:44

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   1 gtgtcccata gtgtttccaa acttggaaag ggcgggggag ggcgggagga tgcggagggc
  61 ggaggtatgc agacaacgag tcagagtttc cccttgaaag cctcaaaagt gtccacgtcc
 121 tcaaaaagaa tggaaccaat ttaagaagcc agccccgtgg ccacgtcccc tccccccattc
 181 gctccctcct ctgcgccccc gcaggctcct cccagctgtg gctgcccggg cccccagccc
 241 cagccctccc attggtggag gccctttttgg aggcacccta gggccaggga aacttttgcc
 301 gtataaatag ggcagatccg ggctttatta ttttagcacc acggcagcag gaggtttcgg
 361 ctaagttgga ggtactggcc acgactgcat gcccgcgccc gccaggtgat acctccgccg
 421 gtgacccagg ggctctgcga cacaaggagt ctgcatgtct aagtgctaga catgctcagc
 481 tttgtggata cgcggacttt gttgctgctt gcagtaacct tatgcctagc aacatgccaa
```

Figure 10 (cont.)

```
 541 tctttacaag aggaaactgt aagaaagggc ccagccggag atagaggacc acgtggagaa
 601 aggggtccac caggcccccc aggcagagat ggtgaagatg gtcccacagg ccctcctggt
 661 ccacctggtc ctcctggccc ccctggtctc ggtgggaact ttgctgctca gtatgatgga
 721 aaaggagttg gacttggccc tggaccaatg ggcttaatgg gacctagagg cccacctggt
 781 gcagctggag ccccaggccc tcaaggtttc caaggacctg ctggtgagcc tggtgaacct
 841 ggtcaaactg gtcctgcagg tgctcgtggt ccagctggcc ctcctggcaa ggctggtgaa
 901 gatggtcacc ctggaaaacc cggacgacct ggtgagagag gagttgttgg accacagggt
 961 gctcgtggtt tccctggaac tcctggactt cctggcttca aaggcattag gggacacaat
1021 ggtctggatg gattgaaggg acagcccggt gctcctggtg tgaagggtga acctggtgcc
1081 cctggtgaaa atggaactcc aggtcaaaca ggagcccgtg ggcttcctgg tgagagagga
1141 cgtgttggtg cccctggccc agctggtgcc cgtggcagtg atggaagtgt gggtcccgtg
1201 ggtcctgctn nnnnnnnnng gtctgctggc cctccaggct tcccaggtgc ccctggcccc
1261 aagggtgaaa ttggagctat tggtaacgct ggtcctgctg gtcccgccgg tccccgtggt
1321 gaagtgggtc ttccaggcct ctccggcccc gttggacctc ctggtaatcc tggagcaaac
1381 ggccttactg gtgccaaggg tgctgctggc cttcccggcg ttgctggggc tcccggcctc
1441 cctggacccc gcggtattcc tggccctgtt ggtgctgccg gtgctactgg tgccagagga
1501 cttgttggtg agcctggtcc agctggctcc aaaggagaga gcggtaacaa gggtgagccc
1561 ggctctgctg ggccccaagg tcctcctggt cccagtggtg aagaaggaaa gagaggccct
1621 aatggggaag ctggatctgc cggccctcca ggacctcctg ggctgagagg tagtcctggt
1681 tctcgtggtc ttcctggagc tgatggcaga gctggcgtca tgggccctcc tggtagtcgt
1741 ggtgcaagtg gccctgctgg agtccgagga cctaatggag atgctggtcg ccctggggag
1801 cctggtctca tgggacccag aggtcttcct ggttcccctg gaaatatcgg ccccgctgga
1861 aaagaaggtc ctgtcggcct ccctggcatc gacggcaggc ctggcccaat tggccccgtt
1921 ggagcaagag gagagcctgg caacattgga ttccctggac ccaaaggccc cactggtgac
1981 cctggcaaaa acggtgataa aggtcatgct ggtcttgctg gtgctcgggg tgctccaggt
2041 cctgatggaa acaatggtgc tcagggacct cctggaccac agggtgttca aggtggaaaa
2101 ggtgaacagg gtcccgctgg tcctccaggc ttccagggtc tgcctggccc ctcaggtccc
2161 gctggtgaag ttggcaaacc aggagaaagg ggtctccatg gtgagtttgg tctccctggt
2221 cctgctggtc caagagggga acgcggtccc ccaggtgaga gtggtgctgc cggtcctact
2281 ggtcctattg gaagccgagg tccttctgga cccccagggc ctgatggaaa caagggtgaa
2341 cctggtgtgg ttggtgctgt gggcactgct ggtccatctg gtcctagtgg actcccagga
2401 gagaggggtg ctgctggcat acctggaggc aagggagaaa agggtgaacc tggtctcaga
2461 ggtgaaattg gtaaccctgg cagagatggt gctcgtggtg ctcatggtgc tgtaggtgcc
2521 cctggtcctg ctggagccac aggtgaccgg ggcgaagctg gggctgctgg tcctgctggt
2581 cctgctggtc ctcggggaag ccctggtgaa cgtggcgagg tcggtcctgc tggccccaac
2641 ggatttgctg gtccggctgg tgctgctggt caaccgggtg ctaaaggaga aagaggaggc
2701 aaagggccta agggtgaaaa cggtgttgtt ggtcccacag gccccgttgg agctgctggc
2761 ccagctggtc caaatggtcc ccccggtcct gctggaagtc gtggtgatgg aggcccccct
2821 ggtatgactg gtttccctgg tgctgctgga cggactggtc ccccaggacc ctctggtatt
2881 tctggccctc ctggtccccc tggtcctgct gggaaagaag ggcttcgtgg tcctcgtggt
2941 gaccaaggtc cagttggccg aactggagaa gtaggtgcag ttggtccccc tggcttcgct
3001 ggtgagaagg gtccctctgg agaggctggt actgctggac ctcctggcac tccaggtcct
3061 cagggtcttc ttggtgctcc tggtattctg ggtctccctg gctcgagagg tgaacgtggt
3121 ctacctggtg ttgctggtgc tgtgggtgaa cctggtcctc ttggcattgc cggccctcct
3181 ggggcccgtg gtcctcctgg tgctgtgggt agtcctggag tcaacggtgc tcctggtgaa
3241 gctggtcgtg atggcaaccc tgggaacgat ggtcccccag gtcgcgatgg tcaacccgga
3301 cacaagggag agcgcggtta ccctggcaat attggtcccg ttggtgctgc aggtgcacct
3361 ggtcctcatg gccccgtggg tcctgctggc aaacatggaa accgtggtga aactggtcct
3421 tctggtcctg ttggtcctgc tggtgctgtt ggcccaagag gtcctagtgg cccacaaggc
3481 attcgtggcg ataagggaga gcccggtgaa aaggggccca gaggtcttcc tggcttcaag
3541 ggacacaatg gattgcaagg tctgcctggt atcgctggtc accatggtga tcaaggtgct
3601 cctggctccg tgggtcctgc tggtcctagg ggccctgctg gtccttctgg ccctgctgga
3661 aaagatggtc gcactggaca tcctggtacg gttggacctg ctggcattcg aggccctcag
3721 ggtcaccaag gccctgctgg ccccccctggt cccccctggcc ctcctggacc tccaggtgta
```

Figure 10 (cont.)

```
3781 agcggtggtg gttatgactt tggttacgat ggagacttct acagggctga ccagcctcgc
3841 tcagcacctt ctctcagacc caaggactat gaagttgatg ctactctgaa gtctctcaac
3901 aaccagattg agacccttct tactcctgaa ggctctagaa agaacccagc tcgcacatgc
3961 cgtgacttga gactcagcca cccagagtgg agcagtggtt actactggat tgaccccaac
4021 caaggatgca ctatggaagc catcaaagta tactgtgatt tccctaccgg cgaaacctgt
4081 atccgggccc aacctgaaaa catcccagcc aagaactggt ataggagctc caaggacaag
4141 aaacacgtct ggctaggaga aactatcaat gctggcagcc agtttgaata taatgttgaa
4201 ggagtgactt ccaaggaaat ggctacccaa cttgccttca tgcgcctgct ggccaactat
4261 gcctctcaga acatcaccta ccactgcaag aacagcattg catacatgga tgaggagact
4321 ggcaacctga aaaaggctgt cattctacag ggctctaatg atgttgaact tgttgctgag
4381 ggcaacagca ggttcactta cactgttctt gtagatggct gctctaaaaa gacaaatgaa
4441 tggggaaaga caatcattga atacaaaaca aataagccat cacgcctgcc cttccttgat
4501 attgcacctt tggacatcgg tggtgctgac catgaattct ttgtggacat tggcccagtc
4561 tgtttcaaat aaatgaactc aatctaaatt aaaaaagaaa gaaatttgaa aaaactttct
4621 ctttgccatt tcttcttctt ctttttaac tgaaagctga atccttccat ttcttctgca
4681 catctacttg cttaaattgt gggcaaaaga gaaaaagaag gattgatcag agcattgtgc
4741 aatacagttt cattaactcc ttcccccgct cccccaaaaa tttgaatttt tttttcaaca
4801 ctcttacacc tgttatggaa aatgtcaacc tttgtaagaa aaccaaaata aaaattgaaa
4861 aataaaaacc ataaacattt gcaccacttg tggcttttga atatcttcca cagagggaag
4921 tttaaaaccc aaacttccaa aggtttaaac tacctcaaaa cactttccca tgagtgtgat
4981 ccacattgtt aggtgctgac ctagacagag atgaactgag gtccttgttt tgttttgttc
5041 ataatacaaa ggtgctaatt aatagtattt cagatacttg aagaatgttg atggtgctag
5101 aagaatttga gaagaaatac tcctgtattg agttgtatcg tgtggtgtat tttttaaaaa
5161 atttgattta gcattcatat tttccatctt attcccaatt aaaagtatgc agattatttg
5221 cccaaagttg tcctcttctt cagattcagc atttgttctt tgccagtctc attttcatct
5281 tcttccatgg ttccacagaa gctttgtttc ttgggcaagc agaaaaatta aattgtacct
5341 attttgtata tgtgagatgt ttaaataaat tgtgaaaaaa atgaaataaa gcatgtttgg
5401 ttttccaaaa gaacatat
```

SEQ Id NO:45

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   1 cagaccacag gaatacctaa tgccttttt ctcttcctgt ctttgtccct cacactacag
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 121 tgggctttgt tctcctgcag gctatggttc atcatgcaaa tagctcctgt gtcagaaatg
 181 ctttttggct tcaaataaca gaaaagctaa caccagcttt atcaataata atatcggtgg
 241 tttacttaag gtgtccagag atggtggaga acaggattgg tttcctcctc aatgtcaagg
 301 actcaaagac tctttctgtg gtagggccac atcctaaacc ctgtatcctg tgattattta
 361 cctgacaggg caaaagagat tttgcagatg caattaaggt taaggacctt gacgtgggaa
 421 gattgtgatt atttacctga cagggcaaaa gagattttgc agatgcaatt aaggttaagg
 481 accttgacgt gggaagatta ttctggatta tctaggtggg cgcaatttga tcacatgggt
 541 ccccagaagt ggagaacctt tcccacctgt agaaagccag agagctggca cctgagaagg
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 661 acctatagag gctaaaaaac agcaaggaaa tggactctcc ccagagcctc cagaggaatg
 721 cagccctgtt gatcacatga tcaccagatg gctgccccag agccaaatgt cgcttcctga
 781 gcaccatact caaaggcagg ggaagtggat ggagggcagg agctccattc ttgtttgcca
 841 ctctcctttt gtcaattggg aaaaaattcc agaaactctg ggagccctcc ccttacattt
 901 cctgggtcat ggggccagcc ctagctgctg gagggactga gaactgctgt tgagcagttt
 961 acctgacggc atctgccatg gcttggcagg aactctggct ttgggagaga gcagcagcaa
1021 ggtattcaag caccacctcc acccagcccc tcccacattt cactcaggac tgagtaaagg
1081 agacactcag atgctactca gatgctggct tcagctaagt attttgcaaa gcctctcgtg
1141 ttcttacaag tttgtggcta tcatgacaaa atggagcagc ctactatatc tacatataca
1201 actatggggg acctagtttt atctcattta ccacaatgtt ttcaatcatt ttttggatga
1261 catatttttt agcctcttct ctaaatgctt cctcaagctt tccttgcctt ccagccactg
1321 caaatgactt gcagtttccc ctacatggca cctgaccctt gtgcctccct ccctctgccc
1381 atggcccaga aagccctttc ctgtgccctc tggcttcctg ataaactcct atcatcttca
```

Figure 10 (cont.)

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1441 agagccagtt cccatgccag ctctccccaa gtgctccact gaggcttccg taacacctct
1501 gttcccacat cgggttgact gtctttgttt tgtcattgct tgctctggct gtgtctccct
1561 cattagactg ggatgccttc aaggtaggga ccctatctgg gtcagcttgg caccccaaag
1621 cgtaccacag cacctgattc tgaggaggct ctcagtagat atctgttgag taaccagaat
1681 gtagggtggt cctgatggtt tctgacattg aatagaaaac agctccctat ttgatcttaa
1741 aataatcact ataacctgga catactgtac tagatgctgt ttttgtctga cttctactct
1801 gtcaatctct ttgcacctcc atttgttcat ctgtgaaatg aagaaaatgc tcatggagtt
1861 cagtgaagat taaatgaatg aatataggta gactgcctaa tctggcactt gccacgcagc
1921 tgacttcaat atagtagctc taatattatg gtccttgagg atcttactgt cttatggccc
1981 agaactgcat ttgattaaag aaggctcccc taaaaaaaga gtcatacata ttccatttgt
2041 cctttcagaa ggccgtgaag catttacact ctttaagaca aattcccatc caaaaatagt
2101 taagatttct aaaatatttt gatgctgaaa gaggtgtgct tcagttgggt ggcaaatttg
2161 cttctatgga agatttttaa tacaggttgt ttctatttta ctttttctgg ctgaaaggat
2221 tttacattta ttcaaagtca aaagggaaaa gaaatccaag aactacagaa gagcagttga
2281 agtgatttat gcttgatttc taaatgcaac ttatgtttat acataattta aaactcaaag
2341 aaagcatgct tatacaatca tgtgcaactt taaactttaa gaactctgga tgaatacatg
2401 gtggcaacag tccatgacac ctgaaaacat catttgtgga gtggcgtaga gttcagtgtt
2461 cgcagtcgca tattacaacc atgtttcaca cagccctgct cggtttgatt ttctccacgt
2521 ggttgataat tgtcttcagt tgctgctaag tgattttgca aatttc
```

SEQ ID NO:46

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   1 gtccccgcgc cagagacgca gccgcgctcc caccacccac acccaccgcg ccctcgttcg
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 121 agccatgtcc accaggtccg tgtcctcgtc ctcctaccgc aggatgttcg gcggcccggg
 181 caccgcgagc cggccgagct ccagccggag ctacgtgact acgtccaccc gcacctacag
 241 cctgggcagc gcgctgcgcc ccagcaccag ccgcagcctc tacgcctcgt ccccgggcgg
 301 cgtgtatgcc acgcgctcct ctgccgtgcg cctgcggagc agcgtgcccg gggtgcggct
 361 cctgcaggac tcggtggact tctcgctggc cgacgccatc aacaccgagt tcaagaacac
 421 ccgcaccaac gagaaggtgg agctgcagga gctgaatgac cgcttcgcca actacatcga
 481 caaggtgcgc ttcctggagc agcagaataa gatcctgctg gccgagctcg agcagctcaa
 541 gggccaaggc aagtcgcgcc tgggggacct ctacgaggag gagatgcggg agctgcgccg
 601 gcaggtggac cagctaacca acgacaaagc ccgcgtcgag gtggagcgcg acaacctggc
 661 cgaggacatc atgcgcctcc gggagaaatt gcaggaggag atgcttcaga gagaggaagc
 721 cgaaaacacc ctgcaatctt tcagacagga tgttgacaat gcgtctctgg cacgtcttga
 781 ccttgaacgc aaagtggaat ctttgcaaga agagattgcc tttttgaaga aactccacga
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 901 tgtttccaag cctgacctca cggctgccct gcgtgacgta cgtcagcaat atgaaagtgt
 961 ggctgccaag aacctgcagg aggcagaaga atggtacaaa tccaagtttg ctgacctctc
1021 tgaggctgcc aaccggaaca atgacgccct gcgccaggca aagcaggagt ccactgagta
1081 ccggagacag gtgcagtccc tcacctgtga agtggatgcc cttaaaggaa ccaatgagtc
1141 cctggaacgc cagatgcgtg aaatggaaga gaactttgcc gttgaagctg ctaactacca
1201 agacactatt ggccgcctgc aggatgagat tcagaatatg aaggaggaaa tggctcgtca
1261 ccttcgtgaa taccaagacc tgctcaatgt taagatggcc cttgacattg agattgccac
1321 ctacaggaag ctgctggaag gcgaggagag caggatttct ctgcctcttc caaacttttc
1381 ctccctgaac ctgagggaaa ctaatctgga ttcactccct ctggttgata cccactcaaa
1441 aaggacactt ctgattaaga cggttgaaac tagagatgga caggttatca acgaaacttc
1501 tcagcatcac gatgaccttg aataaaaatt gcacacactc agtgcagcaa tatattacca
1561 gcaagaataa aaaagaaatc catatcttaa agaaacagct ttcaagtgcc tttctgcagt
1621 ttttcaggag cgcaagatag atttggaata ggaataagct ctagttctta acaaccgaca
1681 ctcctacaag atttagaaaa aagtttacaa cataatctag tttacagaaa aatcttgtgc
1741 tagaatactt tttaaaaggt attttgaata ccattaaaac tgcttttttt tttccagcaa
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SEQ ID NO:47

Figure 10 (cont.)

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 121 accaccatgg atgcagagct ggagtttgcc atccagccca acaccaccgg gaagcagcta
 181 tttgaccagg tggtgaaaac tattggcttg agggaagttt ggttctttgg tctgcagtac
 241 caggacacta aaggtttctc cacctggctg aaactcaata agaaggtgac tgcccaggat
 301 gtgcggaagg aaagcccccc gctctttaag ttccgtgcca agttctaccc tgaggatgtg
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 421 attctcaatg atgatattta ctgcccgcct gagaccgctg tgctgctggc ctcgtatgct
 481 gtccagtcta agtatggcga cttcaataag gaagtgcata agtctggcta cctggccgga
 541 gacaagttgc tcccgcagag agtcctggaa cagcacaaac tcaacaagga ccagtgggag
 601 gagcggatcc aggtgtggca tgaggaacac cgtggcatgc tcagggagga tgctgtcctg
 661 gaatatctga agattgctca agatctggag atgtatggtg tgaactactt cagcatcaag
 721 aacaagaaag gctcagagct gtggctgggg gtggatgccc tgggtctcaa catctatgag
 781 cagaatgaca gactaactcc caagataggc ttcccctgga gtgaaatcag gaacatctct
 841 ttcaatgata agaaatttgt catcaagccc attgacaaaa aagccccgga cttcgtcttc
 901 tatgctcccc ggctgcggat taacaagcgg atcttggcct tgtgcatggg gaaccatgaa
 961 ctatacatgc gccgtcgcaa gcctgatacc attgaggtgc agcagatgaa ggcacaggcc
1021 cgggaggaga agcaccagaa gcagatggag cgtgctatgc tggaaaatga gaagaagaag
1081 cgtgaaatgg cagagaagga gaaagagaag attgaacggg agaaggagga gctgatggag
1141 aggctgaagc agatcgagga acagactaag aaggctcagc aagaactgga agaacagacc
1201 cgtagggctc tggaacttga gcaggaacgg aagcgtgccc agagcgaggc tgaaaagctg
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Figure 10 (cont.)

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3361 aaaatgacat tttgccaaaa ttattaatat aagaagcttt cagtattagt gatgtcatct
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Figure 10 (cont.)

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Figure 10 (cont.)

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 541 ccttgaagat gacattcgct ctgacacatc gttcatgttc cagcgagtgc tggtgtctct
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Figure 10 (cont.)

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 781 tgagacaatg gaagtagact tgggatccca gatacaattg atctgtaatg tcaccggcca
 841 gttgagtgac attgcttact ggaagtggaa tgggtcagta attgatgaag atgacccagt
 901 gctaggggaa gactattaca gtgtggaaaa tcctgcaaac aaaagaagga gtaccctcat
 961 cacagtgctt aatatatcgg aaattgaaag tagattttat aaacatccat ttacctgttt
1021 tgccaagaat acacatggta tagatgcagc atatatccag ttaatatatc cagtcactaa
1081 tttccagaag cacatgattg gtatatgtgt cacgttgaca gtcataattg tgtgttctgt
1141 tttcatctat aaaatcttca agattgacat tgtgctttgg tacagggatt cctgctatga
1201 ttttctccca ataaaagctt cagatggaaa gacctatgac gcatatatac tgtatccaaa
1261 gactgttggg gaagggtcta cctctgactg tgatattttt gtgtttaaag tcttgcctga
1321 ggtcttggaa aaacagtgtg gatataagct gttcatttat ggaagggatg actacgttgg
```

Figure 10 (cont.)

```
1381 ggaagacatt gttgaggtca ttaatgaaaa cgtaaagaaa agcagaagac tgattatcat
1441 tttagtcaga gaaacatcag gcttcagctg gctgggtggt tcatctgaag agcaaatagc
1501 catgtataat gctcttgttc aggatggaat taaagttgtc ctgcttgagc tggagaaaat
1561 ccaagactat gagaaaatgc cagaatcgat taaattcatt aagcagaaac atggggctat
1621 ccgctggtca ggggactttа cacagggacc acagtctgca aagacaaggt tctggaagaa
1681 tgtcaggtac cacatgccag tccagcgacg gtcaccttca tctaaacacc agttactgtc
1741 accagccact aaggagaaac tgcaaagaga ggctcacgtg cctctcgggt agcatggaga
1801 agttgccaag agttctttag gtgcctcctg tcttatggcg ttgcaggcca ggttatgcct
1861 catgctgact tgcagagttc atggaatgta actatatcat cctttatccc tgaggtcacc
1921 tggaatcaga ttattaaggg aataagccat gacgtcaata gcagcccagg gcacttcaga
1981 gtagagggct tgggaagatc ttttaaaaag gcagtaggcc cggtgtggtg gctcacgcct
2041 ataatcccag cactttggga ggctgaagtg ggtggatcac cagaggtcag gagttcgaga
2101 ccagcccagc caacatggca aaaccccatc tctactaaaa atacaaaaat gagctaggca
2161 tggtggcaca cgcctgtaat cccagctaca cctgaggctg aggcaggaga attgcttgaa
2221 ccggggagac ggaggttgca gtgagccgag tttgggccac tgcactctag cctggcaaca
2281 gagcaagact ccgtctcaaa aaaagggcaa taaatgccct ctctgaatgt ttgaactgcc
2341 aagaaaaggc atggagacag cgaactagaa gaaagggcaa gaaggaaata gccaccgtct
2401 acagatggct tagttaagtc atccacagcc caagggcggg gctatgcctt gtctggggac
2461 cctgtagagt cactgaccct ggagcggctc tcctgagagg tgctgcaggc aaagtgagac
2521 tgacacctca ctgaggaagg gagacatatt cttggagaac tttccatctg cttgtatttt
2581 ccatacacat ccccagccag aagttagtgt ccgaagaccg aattttattt tacagagctt
2641 gaaaactcac ttcaatgaac aaagggattc tccaggattc caaagttttg aagtcatctt
2701 agctttccac aggagggaga gaacttaaaa aagcaacagt agcagggaat tgatccactt
2761 cttaatgctt tcctccctgg catgaccatc ctgtcctttg ttattatcct gcattttacg
2821 tctttggagg aacagctccc tagtggcttc ctccgtctgc aatgtccctt gcacagccca
2881 cacatgaacc atccttccca tgatgccgct cttctgtcat cccgctcctg ctgaaacacc
2941 tcccaggggc tccacctgtt caggagctga agcccatgct ttcccaccag catgtcactc
3001 ccagaccacc tccctgccct gtcctccagc ttcccctcgc tgtcctgctg tgtgaattcc
3061 caggttggcc tggtggccat gtcgcctgcc cccagcactc ctctgtctct gctcttgcct
3121 gcacccttcc tcctcctttg cctaggaggc cttctcgcat tttctctagc tgatcagaat
3181 tttaccaaaa ttcagaacat cctccaattc cacagtctct gggagacttt ccctaagagg
3241 cgacttcctc tccagccttc tctctctggt caggcccact gcagagatgg tggtgagcac
3301 atctgggagg ctggtctccc tccagctgga attgctgctc tctgagggag aggctgtggt
3361 ggctgtctct gtccctcact gccttccagg agcaatttgc acatgtaaca tagatttatg
3421 taatgcttta tgtttaaaaa cattccccaa ttatcttatt taatttttgc aattattcta
3481 attttatata tagagaaagt gacctatttt ttaaaaaaat cacactctaa gttctattga
3541 acctaggact tgagcctcca tttctggctt ctagtctggt gttctgagta cttgatttca
3601 ggtcaataac ggtccccccc cactccacac tggcacgttt gtgagaagaa atgacatttt
3661 gctaggaagt gaccgagtct aggaatgctt ttattcaaga caccaaattc caaacttcta
3721 aatgttggaa ttttcaaaaa ttgtgtttag attttatgaa aaactcttct actttcatct
3781 attctttccc tagaggcaaa catttcttaa aatgtttcat tttcattaaa aatgaaagcc
3841 aaatttatat gccaccgatt gcaggacaca agcacagttt taagagttgt atgaacatgg
3901 agaggacttt tggtttttat atttctcgta tttaatatgg gtgaacacca acttttattt
3961 ggaataataa ttttcctcct aaacaaaaac acattgagtt taagtctctg actcttgcct
4021 ttccacctgc tttctcctgg gcccgctttg cctgcttgaa ggaacagtgc tgttctggag
4081 ctgctgttcc aacagacagg gcctagcttt catttgacac acagactaca gccagaagcc
4141 catggagcag ggatgtcacg tcttgaaaag cctattagat gttttacaaa tttaattttg
4201 cagattattt tagtctgtca tccagaaaat gtgtcagcat gcatagtgct aagaaagcaa
4261 gccaatttgg aaacttaggt tagtgacaaa attggccaga gagtgggggt gatgatgacc
4321 aagaattaca agtagaatgg cagctggaat ttaaggaggg acaagaatca atggataagc
4381 gtgggtggag gaagatccaa acagaaaagt gcaaagttat tccccatctt ccaagggttg
4441 aattctggag gaagaagaca cattcctagt tccccgtgaa cttcctttga cttattgtcc
4501 ccactaaaac aaaacaaaaa acttttaatg ccttccacat taattagatt ttcttgcagt
4561 ttttttatgg catttttttа aagatgccct aagtgttgaa gaagagtttg caaatgcaac
```

Figure 10 (cont.)

```
4621 aaaatattta attaccggtt gttaaaactg gtttagcaca atttatattt tccctctctt
4681 gcctttctta tttgcaataa aaggtattga gccatttttt aaatgacatt tttgataaat
4741 tatgtttgta ctagttgatg aaggagtttt ttttaacctg tttatataat tttgcagcag
4801 aagccaaatt ttttgtatat taaagcacca aattcatgta cagcatgcat cacggatcaa
4861 tagactgtac ttattttcca ataaaatttt caaactttgt actgtta

SEQ ID NO:54
   1 ccctgcactc tcgctctcct gccccacccc gaggtaaagg gggcgactaa gagaagatgg
  61 tgttgctcac cgcggtcctc ctgctgctgg ccgcctatgc ggggccggcc cagagcctgg
 121 gctccttcgt gcactgcgag ccctgcgacg agaaagccct ctccatgtgc ccccccagcc
 181 ccctgggctg cgagctggtc aaggagccgg gctgcggctg ctgcatgacc tgcgccctgg
 241 ccgaggggca gtcgtgcggc gtctacaccg agcgctgcgc ccaggggctg cgctgcctcc
 301 cccggcagga cgaggagaag ccgctgcacg ccctgctgca cggccgcggg gtttgcctca
 361 acgaaaagag ctaccgcgag caagtcaaga tcgagagaga ctcccgtgag cacgaggagc
 421 ccaccacctc tgagatggcc gaggagacct actcccccaa gatcttccgg cccaaacaca
 481 cccgcatctc cgagctgaag gctgaagcag tgaagaagga ccgcagaaag aagctgaccc
 541 agtccaagtt tgtcgggggа gccgagaaca ctgcccaccc ccggatcatc tctgcacctg
 601 agatgagaca ggagtctgag cagggccccт gccgcagaca catggaggct tccctgcagg
 661 agctcaaagc cagcccacgc atggtgcccc gtgctgtgta cctgcccaat tgtgaccgca
 721 aaggattcta caagagaaag cagtgcaaac cttcccgtgg ccgcaaacgt ggcatctgct
 781 ggtgcgtgga caagtacggg atgaagctgc caggcatgga gtacgttgac ggggacttтc
 841 agtgccacac cttcgacagc agcaacgttg agtgatgcgt ccccccccaa cctttccctc
 901 acccccтccc acccccagcc ccgactccag ccagcgcctc cctccacccc aggacgccac
 961 tcatttcatc tcatttaagg gaaaaatata tatctatcta tttg

SEQ ID NO:55
   1 cgcagcgggt cctctctatc tagctccagc ctctcgcctg cgccccactc cccgcgtccc
  61 gcgtcctagc cgaccatggc cgggcccctg cgcgccccgc tgctcctgct ggccatcctg
 121 gccgtggccc tggccgtgag ccccgcggcc ggctccagtc ccggcaagcc gccgcgccta
 181 gtgggaggcc ccatggacgc cagcgtggag gaggagggtg tgcggcgtgc actggacttt
 241 gccgtcggcg agtacaacaa agccagcaac gacatgtacc acagccgcgc gctgcaggtg
 301 gtgcgcgccc gcaagcagat cgtagctggg gtgaactact tcttggacgt ggagctgggc
 361 cgaaccacgt gtaccaagac ccagcccaac ttggacaact gccccttcca tgaccagcca
 421 catctgaaaa ggaaagcatt ctgctctttc cagatctacg ctgtgccttg gcagggcaca
 481 atgaccttgt cgaaatccac ctgtcaggac gcctaggggt ctgtaccggg ctggcctgtg
 541 cctatcacct cttatgcaca cctcccaccc cctgtattcc caccctgga ctggtggccc
 601 ctgccttggg gaaggtctcc ccatgtgcct gcaccaggag acagacagag aaggcagcag
 661 gcggcctttg ttgctcagca aggggctctg ccctccctcc ttccttcttg cttctcatag
 721 ccccggtgtg cggtgcatac acccccacct cctgcaataa aatagtagca tc

SEQ ID NO:56

   1 gaaagatgga tcactccagc tcaaagagaa catgtgggaa tgaaaggaca ggctgggccc
  61 aaaggagaaa agggtgatgc tggggaggag cttcctggcc ctcctgaacc ttctgggcct
 121 gttggaccca cggcaggagc agaagcagag ggctctggcc taggctgggg ctcggacgtc
 181 ggctctggct ctggtgacct ggtgggcagt gagcagctgc tgagaggtcc tccaggaccc
 241 ccagggccac ctggcttacc tgggattcca ggaaaaccag gaactgatgt tttcatggga
 301 ccccctggat ctcctggaga ggatggacct gctggtgaac ctgggccccc gggccctgag
 361 ggacagcctg gagttgatgg agccaccggc cttcccggga tgaaagggga gaagggagca
 421 agagggccta atggctcagt tggtgaaaag ggtgaccctg gcaacagagg cttacctgga
 481 cccccgggga aaaagggaca agctggccct cctggggtca tgggaccccc agggcctcct
 541 ggacccccтg ggcccccagg ccctggatgc acaatgggac ttggattcga ggataccgaa
 601 ggctctggaa gcacccagct attgaatgaa cccaaactct ccagaccaac ggctgcaatt
```

Figure 10 (cont.)

```
 661  ggtctcaaag gagagaaagg agaccgggga cccaagggag aaagggggat ggatggagcc
 721  agtattgtgg gacccccctgg gccgagaggg ccacctgggc acatcaaggt cttgtctaat
 781  tccttgatca atatcaccca tggattcatg aatttctcgg acattcctga gctggtgggg
 841  cctccggggc cggacgggtt gcctgggctg ccaggatttc cagggtccta gaggaccaaa
 901  aggtgacact ggtttacctg gctttccagg actaaaagga gaacagggcg agaagggaga
 961  gccgggtgcc atcctgacag aggacattcc tctggaaagg ctgatgggga aaaagggtga
1021  acctggaatg catggagccc caggaccaat ggggcccaaa ggaccaccag gacataaagg
1081  agaatttggc cttcccgggc gacctggtcg cccaggactg aatggcctca agggtaccaa
1141  aggagatcca ggggtcatta tgcagggccc acctggctta cctggccctc caggcccccc
1201  tgggccacct ggagctgtga ttaacatcaa aggagccatt ttcccaatac ccgtccgacc
1261  acactgcaaa atgccagttg atactgctca tcctgggagt ccagagctca tcacttttca
1321  cggtgttaaa ggagagaaag gatcctgggg tcttcctggc tcaaagggag aaaaaggcga
1381  ccagggagcc cagggaccac caggtcctcc acttgatcta gcttacctga gacactttct
1441  gaacaacttg aaggggggaga atggagacaa ggggttcaaa ggtgaaaaag gagaaaaagg
1501  agacattaat ggcagcttcc ttatgtctgg gcctccaggc ctgcccggaa atccaggccc
1561  ggctggccaa aaaggggaga cagtcgttgg gccccaagga cccccaggtg ctcctggtct
1621  gcctgggcca cctggctttg gaagacctgg tgatcctggg ccaccggggc ccccggggcc
1681  accaggacct ccagctatcc tgggagcagc tgtggccctt ccaggtcccc ctggccctcc
1741  aggacagcca gggcttcccg gatccagaaa cctggtcaca gcattcagca acatggatga
1801  catgctgcag aaagcgcatt tggttataga aggaacattc atctacctga gggacagcac
1861  tgagtttttc attcgtgtta gagatggctg gaaaaaatta cagctgggag aactgatccc
1921  cattcctgcc gacagccctc cacccccctgc gctttccagc aacccacatc agcttctgcc
1981  tccaccaaac cctatttcaa gtgccaatta tgagaagcct gctctgcatt tggctgctct
2041  gaacatgcca ttttctgggg acattcgagc tgattttcag tgcttcaagc aggccagagc
2101  tgcaggactg ttgtccacct accgagca
```

SEQ ID NO:57

```
   1  tagaaattgt taattttaac aatccagagc aggccaacga ggctttgctc tcccgacccg
  61  aactaaaggt ccctcgctcc gtgcgctgct acgagcggtg tctcctgggg ctccaatgca
 121  gcgagctgtg cccgaggggt tcggaaggcg caagctgggc agcgacatgg ggaacgcgga
 181  gcgggctccg gggtctcgga gctttgggcc agtacccacg ctgctgctgc tcgccgcggc
 241  gctactggcc gtgtcggacg cactcgggcg cccctccgag gaggacgagg agctagtggt
 301  gccggagctg gagcgcgccc cgggacacgg gaccacgcgc ctccgcctgc acgcctttga
 361  ccagcagctg gatctggagc tgcggcccga cagcagcttt ttggcgcccg gcttcacgct
 421  ccagaacgtg gggcgcaaat ccgggtccga gacgccgctt ccggaaaccg acctggcgca
 481  ctgcttctac tccggcaccg tgaatggcga tcccagctcg gctgccgccc tcagcctctg
 541  cgagggcgtg cgcggcgcct tctacctgct gggggaggcg tatttcatcc agccgctgcc
 601  cgccgccagc gagcgcctcg ccaccgccgc cccaggggag aagccgccgg caccactaca
 661  gttccacctc ctgcggcgga atcggcaggg cgacgtcggc ggcacgtgcg gggtcgtgga
 721  cgacgagccc cggccgactg ggaaagcgga gaccgaagac gaggacgaag ggactgaggg
 781  cgaggacgaa ggggctcagt ggtcgccgca ggacccggca ctgcaaggcg taggacagcc
 841  cacaggaact ggaagcataa gaaagaagcg atttgtgtcc agtcaccgct atgtggaaac
 901  catgcttgtg gcagaccagt cgatggcaga attccacggc agtggtctaa agcattacct
 961  tctcacgttg ttttcggtgg cagccagatt gtacaaacac cccagcattc gtaattcagt
1021  tagcctggtg gtggtgaaga tcttggtcat ccacgatgaa cagaaggggc cggaagtgac
1081  ctccaatgct gccctcactc tgcggaactt ttgcaactgg cagaagcagc acaacccacc
1141  cagtgaccgg gatgcagagc actatgacac agcaattctt ttcaccagac aggacttgtg
1201  tgggtcccag acatgtgata ctcttgggat ggctgatgtt ggaactgtgt gtgatccgag
1261  cagaagctgc tccgtcatag aagatgatgg tttacaagct gccttcacca cagcccatga
1321  attaggccac gtgtttaaca tgccacatga tgatgcaaag cagtgtgcca gccttaatgg
1381  tgtgaaccag gattcccaca tgatggcgtc aatgctttcc aacctggacc acagccagcc
1441  ttggtctcct tgcagtgcct acatgattac atcatttctg gataatggtc atggggaatg
1501  tttgatggac aagcctcaga atcccataca gctcccaggc gatctccctg gcacctcgta
1561  cgatgccaac cggcagtgcc agtttacatt tggggaggac tccaaacact gccccgatgc
```

Figure 10 (cont.)

```
1621 agccagcaca tgtagcacct tgtggtgtac cggcacctct ggtggggtgc tggtgtgtca
1681 aaccaaacac ttcccgtggg cggatggcac cagctgtgga gaagggaaat ggtgtatcaa
1741 cggcaagtgt gtgaacaaaa ccgacagaaa gcattttgat acgccttttc atggaagctg
1801 gggaatgtgg gggccttggg gagactgttc gagaacgtgc ggtggaggag tccagtacac
1861 gatgagggaa tgtgacaacc cagtcccaaa gaatggaggg aagtactgtg aaggcaaacg
1921 agtgcgctac agatcctgta accttgagga ctgtccagac aataatggaa aaacctttag
1981 agaggaacaa tgtgaagcac acaacgagtt ttcaaaagct tcctttggga gtgggcctgc
2041 ggtggaatgg attcccaagt acgctggcgt ctcaccaaag gacaggtgca agctcatctg
2101 ccaagccaaa ggcattggct acttcttcgt tttgcagccc aaggttgtag atggtactcc
2161 atgtagccca gattccacct ctgtctgtgt gcaaggacag tgtgtaaaag ctggttgtga
2221 tcgcatcata gactccaaaa agaagtttga taaatgtggt gtttgcgggg gaaatggatc
2281 tacttgtaaa aaaatatcag gatcagttac tagtgcaaaa cctggatatc atgatatcat
2341 cacaattcca actggagcca ccaacatcga agtgaaacag cggaaccaga ggggatccag
2401 gaacaatggc agctttcttg ccatcaaagc tgctgatggc acatatattc ttaatggtga
2461 ctacactttg tccaccttag agcaagacat tatgtacaaa ggtgttgtct tgaggtacag
2521 cggctcctct gcggcattgg aaagaattcg cagctttagc cctctcaaag agcccttgac
2581 catccaggtt cttactgtgg gcaatgccct tcgacctaaa attaaataca cctacttcgt
2641 aaagaagaag aaggaatctt tcaatgctat ccccactttt tcagcatggg tcattgaaga
2701 gtggggcgaa tgttctaagt catgtgaatt gggttggcag agaagactgg tagaatgccg
2761 agacattaat ggacagcctg cttccgagtg tgcaaaggaa gtgaagccag ccagcaccag
2821 accttgtgca gaccatccct gcccccagtg gcagctgggg gagtggtcat catgttctaa
2881 gacctgtggg aagggttaca aaaaaagaag cttgaagtgt ctgtcccatg atggaggggt
2941 gttatctcat gagagctgtg atcctttaaa gaaacctaaa catttcatag acttttgcac
3001 aatggcagaa tgcagttaag tggtttaagt ggtgttagct ttgagggcaa ggcaaagtga
3061 ggaagggctg gtgcagggaa agcaagaagg ctggagggat ccagcgtatc ttgccagtaa
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3181 cagagtaaac tgccagttgc aaatttgata ggatagttag tgaggattat taacctctga
3241 gcagtgatat agcataataa agcccccggc attattatta ttatttcttt tgttacatct
3301 attacaagtt tagaaaaaac aaagcaattg tcaaaaaaag ttagaactat tacaacccct
3361 gtttcctggt acttatcaaa tacttagtat catgggggtt gggaaatgaa aagtaggaga
3421 aaagtgagat tttactaaga cctgttttac tttacctcac taacaatggg gggagaaagg
3481 agtacaaata ggatctttga ccagcactgt ttatggctgc tatggtttca gagaatgttt
3541 atacattatt tctaccgaga attaaaactt cagattgttc aacatgagag aaaggctcag
3601 caacgtgaaa taacgcaaat ggcttcctct ttccttttct ggaccatctc agtctttatt
3661 tgtgtaattc attttgagga aaaaacaact ccatgtattt attcaagtgc attaaagtct
3721 acaatggaaa aaaagcagtg aagcattaga tgctggtaaa agctagagga gacacaatga
3781 gcttagtacc tccaacttcc tttctttcct accatgtaac cctgctttgg gaatatggat
3841 gtaaagaagt aacttgtgtc tcatgaaaat cagtacaatc acacaaggag gatgaaacgc
3901 cggaacaaaa atgaggtgtg tagaacaggg tcccacaggt ttggggacat tgagatcact
3961 tgtcttgtgg tggggaggct gctgaggggt agcaggtcca tctccagcag ctggtccaac
4021 agtcgtatcc tggtgaatgt ctgttcagct cttctgtgag aatatgattt tttccatatg
4081 tatatagtaa aatatgttac tataaattac atgtacttta taagtattgg tttgggtgtt
4141 ccttccaaga aggactatag ttagtaataa atgcctataa taacatattt atttttatac
4201 atttatttct aatgaaaaaa acttttaaat tatatcgctt ttgtggaagt gcatataaaa
4261 tagagtattt atacaatata tgttactaga aataaaagaa cacttttgg
```

SEQ ID NO:58

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 121 ccacctgagc tccatgagct cgctgggcgg cccggccgct ttctcggcgc gctgggcgca
 181 ggaggcctac aagaaggaga gcgccaagga ggcgggcgcg gccgcggtgc cggcgccggt
 241 gcccgcagcc accgagccgc cgcccgtgct gcacctgccc gccatccagc cgccgccgcc
 301 cgtgctgccc gggcccttct tcatgccgtc cgaccgctcc accgagcgct gcgagaccgt
 361 actggaaggc gagaccatct cgtgcttcgt ggtgggaggc gagaagcgcc tgtgtctgcc
```

Figure 10 (cont.)

```
 421 gcagattctc aactcggtgc tgcgcgactt ctcgctgcag cagatcaacg cggtgtgcga
 481 cgagctccac atctactgct cgcgctgcac ggccgaccag ctggagatcc tcaaagtcat
 541 gggcatcctg cccttctcgg cgccctcgtg cgggctcatc accaagacgg acgccgagcg
 601 cctgtgcaac gcgctgctct acggcggcgc ctacccgccg ccctgcaaga aggagctggc
 661 cgccagcctg gcgctgggcc tggagctcag cgagcgcagc gtccgcgtgt accacgagtg
 721 cttcggcaag tgtaaggggc tgctggtgcc cgagctctac agcagcccga gcgccgcctg
 781 catccagtgc ctggactgcc gcctcatgta cccgccgcac aagttcgtgg tgcactcgca
 841 caaggccctg gagaaccgga cctgccactg gggcttcgac tcggccaact ggcgggccta
 901 catcctgctg agccaggatt acacgggcaa ggaggagcag gcgcgcctcg gccgctgcct
 961 ggacgacgtg aaggagaaat tcgactatgg caacaagtac aagcggcggg tgccccgggt
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1201 cgcagtgtca gcgagtgaga aagagctctc cccacacctc ccggccctca tccgagacag
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1861 ggccacggag gccaagcgta acctgcggaa ggagatcgag cgtctccgcg ccgagaacga
1921 gaagaagatg aaagaggcca acgagtcacg gctgcgcctg aagcgggagc tggagcaggc
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2461 gacggggcca gctcggcggc ctgctggtcc tctgcttgct ggaacattct aacatttaca
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3001 acctgcgtcg ggtggtgacc gtggctggcg gtcacgccct cagcccctcc gggcacacgt
3061 gccgcctgac cgggcgaccc ttttcagttc ggcaaacgtc gctcccttca ttttgggact
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3301 gcttagaaac cccctctggt gcttggttga acaagggaat cacaagaaaa cgaaaatgca
3361 aaaactgaac ttcgggggtc gttctgtgcc ttccagcatc ttgtacagca aatcctgact
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3481 ataagttc
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SEQ Id NO:59

Figure 10 (cont.)

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 121 gcctgcacgc ttctcagtgt tccccgcgcc ccgcatgtaa cccggccagg ccccgcaac
 181 tgtgtccect gcagctccag ccccgggctg catccccccg ccccgacacc agctctccag
 241 cctgctcgtc caggatggcc gcggccaagg ccgagatgca gctgatgtcc ccgctgcaga
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 421 agggcagcgg cagcaacagc agcagcagca gcagcggggg cggtggaggc ggcgggggcg
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 541 cctacgagca cctgaccgca gagtcttttc ctgacatctc tctgaacaac gagaaggtgc
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 661 gcttttccct ggagcctgca cccaacagtg gcaacacctt gtggcccgag cccctcttca
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 781 ctccagcggc ctcctccgcc tccgcctccc agagcccacc cctgagctgc gcagtgccat
 841 ccaacgacag cagtcccatt tactcagcgg cacccacctt ccccacgccg aacactgaca
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 961 cgcctcctgc ctaccctgcc gccaagggtg gcttccaggt tcccatgatc cccgactacc
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1081 gcctggagag ccgcacccag cagccttcgc taacccctct gtctactatt aaggcctttg
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1621 cctcttatcc atccccggcc accacctcat acccatcccc tgtgcccacc tccttctcct
1681 ctcccggctc ctcgacctac ccatcccctg tgcacagtgg cttcccctcc ccgtcggtgg
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2401 aagaaaagcc aagcaaacca atggtgatcc tctattttgt gatgatgctg tgacaataag
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2761 ctttgggagc aaaataagga agagggctga gctgagcttc ggttctccag aatgtaagaa
2821 aacaaaatct aaaacaaaat ctgaactctc aaaagtctat ttttttaact gaaaatgtaa
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SEQ ID NO:60

Figure 10 (cont.)

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 241 ggagaccgta gctgaagctg attctgtaca gcgggacagc gctttctgcc cctgggggag
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 421 gacgttgctc cttccccgag cttccccgga cagcgtactt tgaggactcg ctcagctcac
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3121 ccagacccat tccttagcta tttatccctt tcctggtttc cgaaaggcaa ttatatctat
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Figure 10 (cont.)

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3661 tggttctctt tttgtatttt gcatctgacc ccgggggget gggacagatt ggcaatgggc
3721 cgtcccctct ccccttggtt ctgcactgtt gccaataaaa agctcttaaa aacgc

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 421 agtctaagag taggatgtct gctgaagtca tccatcaggt tgaagaagca cttgatacag
 481 atgagaagga gatgctgctc tttttgtgcc gggatgttgc tatagatgtg gttccaccta
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```

Figure 10 (cont.)

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 541 ggaggagggg acaagtcgtc ggagtccggg cggccaagac ccgccgccgg ccggccactg
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1861 cacggccaac atgctcaggg aacaggtggc acagcttaaa cagaaagtca tgaaccacgt
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1981 cgggggctga ggggcaacga agaaaaaaaa taacacagag agacagactt gagaacttga
2041 caagttgcga cggagagaaa aaagaagtgt ccgagaacta aagccaaggg tatccaagtt
2101 ggactgggtt gcgtcctgac ggcgccccca gtgtgcacga gtgggaagga cttggcgcgc
2161 cctcccttgg cgtggagcca gggagcggcc gcctgcgggc tgccccgctt tgcggacggg
2221 ctgtccccgc gcgaacggaa cgttggactt ttcgttaaca ttgaccaaga actgcatgga
2281 cctaacattc gatctcattc agtattaaag gggggagggg gaggggttta caaactgcaa
2341 tagagactgt agattgcttc tgtagtactc cttaagaaca caaagcgggg ggagggttgg
2401 ggaggggcgg caggagggag gtttgtgaga gcgaggctga gcctacagat gaactctttc
2461 tggcctgcct tcgttaactg tgtatgtaca tatatatatt ttttaatttg atgaaagctg
2521 attactgtca ataaacagct tcatgccttt gtaagttatt tcttgtttgt ttgtttgggt
2581 atcctgccca gtgttgtttg taaataagag atttggagca ctctgagttt accatttgta
2641 ataaagtata taatttttct atgttttgtt tctgaaaatt ccagaaagga tatttaagaa
2701 aatacaataa actattggaa agtactcccc taacctcttt tctgcatcat ctgtagatac
2761 tagctatcta ggtggagttg aaagagttaa gaatgtcgat taaaatcact ctcagtgctt
2821 cttactatta agcagtaaaa actgttctct attagacttt agaaataaat gtacctgatg
2881 tacctgatgc tatggtcagg ttatactcct cctcccccag ctatctatat ggaattgctt
2941 accaaaggat agtgcgatgt ttcaggaggc tggaggaagg ggggttgcag tggagaggga
3001 cagcccactg agaagtcaaa catttcaaag tttggattgt atcaagtggc atgtgctgtg
3061 accatttata atgttagtag aaattttaca ataggtgctt attctcaaag caggaattgg
3121 tggcagattt tacaaaagat gtatccttcc aatttggaat cttctctttg acaattccta
3181 gataaaaaga tggcctttgc ttatgaatat ttataacagc attcttgtca caataaatgt
3241 attcaaatac caat
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SEQ ID NO:63

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   1 gtggagctac cgccaccgcc gccgccgatt ccggagccgg ggtagtcgcc gccgccgccg
  61 ccgctgcagc cactgcaggc accgctgccg ccgcctgagt agtgggctta ggaaggaaga
 121 ggtcatctcg ctcggagctt cgctcggaag ggtctttgtt ccctgcagcc ctcccacggg
 181 aatgacaatg gataaaagtg agctggtaca gaaagccaaa ctcgctgagc aggctgagcg
```

Figure 10 (cont.)

```
241 atatgatgat atggctgcag ccatgaaggc agtcacagaa caggggcatg aactctccaa
301 cgaagagaga aatctgctct ctgttgccta caagaatgtg gtaggcgccc gccgctcttc
361 ctggcgtgtc atctccagca ttgagcagaa aacagagagg aatgagaaga agcagcagat
421 gggcaaagag taccgtgaga agatagaggc agaactgcag gacatctgca atgatgttct
481 ggagctgttg gacaaatatc ttattcccaa tgctacacaa ccagaaagta aggtgttcta
541 cttgaaaatg aaaggagatt attttaggta tctttctgaa gtggcatctg gagacaacaa
601 acaaaccact gtgtcgaact cccagcaggc ttaccaggaa gcatttgaaa ttagtaagaa
661 agaaatgcag cctacacacc caattcgtct tggtctggca ctaaatttct cagtctttta
721 ctatgagatt ctaaactctc ctgaaaaggc ctgtagcctg gcaaaaacgg catttgatga
781 agcaattgct gaattggata cgctgaatga agagtcttat aaagacagca ctctgatcat
841 gcagttactt agggacaatc tcactctgtg gacatcggaa aaccagggag acgaaggaga
901 cgctggggag ggagagaact aatgtttctc gtgctttgtg atctgttcag tgtcactctg
961 taccctcaac atatatccct tgtgcgat
```

SEQ ID NO:64

```
1 gtgccgctcc ttggtggggg ctgttcatgg cggttccggg gtctccaaca ttttcccgg
61 ctgtggtcct aaatctgtcc aaagcagagg cagtggagct tgaggttctt gctggtgtga
121 aatgactgag tacaaactgg tggtggttgg agcaggtggt gttgggaaaa gcgcactgac
181 aatccagcta atccagaacc actttgtaga tgaatatgat cccaccatag aggattctta
241 cagaaaacaa gtggttatag atggtgaaac ctgtttgttg gacatactgg atacagctgg
301 acaagaagag tacagtgcca tgagagacca atacatgagg acaggcgaag gcttcctctg
361 tgtatttgcc atcaataata gcaagtcatt tgcggatatt aacctctaca gggagcagat
421 taagcgagta aaagactcgg atgatgtacc tatggtgcta gtgggaaaca agtgtgattt
481 gccaacaagg acagttgata caaaacaagc ccacgaactg gccaagagtt acgggattcc
541 attcattgaa acctcagcca agaccagaca gggtgttgaa gatgcttttt acacactggt
601 aagagaaata cgccagtacc gaatgaaaaa actcaacagc agtgatgatg ggactcaggg
661 ttgtatggga ttgccatgtg tggtgatgta acaagatact tttaaagttt tgtcagaaaa
721 gagccacttt caagctgcac tgacaccctg gtcctgactt ccctggagga gaagtattcc
781 tgttgctgtc ttcagtctca cagagaagct cctgctactt ccccagctct cagtagttta
841 gtacaataat ctctatttga gaagttctca gaataactac ctcctcactt ggctgtctga
901 ccagagaatg cacctcttgt tactccctgt tatttttctg ccctgggttc ttccacagca
961 caaacacacc tctgccaccc caggtttttc atctgaaaag cagttcatgt ctgaaacaga
1021 gaaccaaacc gcaaacgtga aattctattg aaaacagtgt cttgagctct aaagtagcaa
1081 ctgctggtga ttttttttct ctttttactg ttgaacttag aactatgcta atttttggag
1141 aaatgtcata aattactgtt ttgccaagaa tatagttatt attgctgttt ggtttgttta
1201 taatgttatc ggctctattc tctaaactgg catctgctct agattcataa atacaaaaat
1261 gaatactgaa ttttgagtct atcctagtct tcacaacttt gacgtaatta aatccaactt
1321 tcacagtgaa gtgccttttt cctagaagtg gtttgtagac ttcctttata atatttcagt
1381 ggaatagatg tctcaaaaat ccttatgcat gaaatgaatg tctgagatac gtctgtgact
1441 tatctaccat tgaaggaaag ctatatctat ttgagagcag atgccatttt gtacatgtat
1501 gaaattggtt ttccagaggc ctgttttggg gctttcccag gagaaagatg aaactgaaag
1561 cacatgaata atttcactta ataattttta cctaatctcc actttttca taggttacta
1621 cctatacaat gtatgtaatt tgtttccccc agcttactga taaacctaat attcaatgaa
1681 cttccatttg tattcaaatt tgtgtcatac cagaaagctc tacatttgca gatgttcaaa
1741 tattgtaaaa ctttggtgca ttgttattta atagctgtga tcagtgattt tcaaacctca
1801 aatatagtat attaacaaat tacattttca ct
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SEQ ID NO:65

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61 agtgcctcct ctccagtcaa ctgccagtgg gacttctatg ccccttggtc agaatgcaat
121 ggctgtacca agactcagac tcgcaggcgg tcagttgctg tgtatgggca gtatggaggc
181 cagccttgtg ttggaaatgc ttttgaaaca cagtcctgtg aacctacaag aggatgtcca
241 acagaggagg gatgtggaga gcgtttcagg tgcttttcag gtcagtgcat cagcaaatca
301 ttggtttgca atggggattc tgactgtgat gaagacagtg ctgatgaaga cagatgtgag
```

Figure 10 (cont.)

```
 361 gactcagaaa ggagaccttc ctgtgatatc gataaacctc ctcctaacat agaacttact
 421 ggaaatggtt acaatgaact cactggccag tttaggaaca gagtcatcaa taccaaaagt
 481 tttggtggtc aatgtagaaa ggtgtttagt ggggatggaa aagatttcta caggctgagt
 541 ggaaatgtcc tgtcctatac attccaggtg aaaataaata atgattttaa ttatgaattt
 601 tacaatagta cttggtctta tgtaaaacat acgtcgacag aacacacatc atctagtcgg
 661 aagcgctcct tttttagatc ttcatcatct tcttcacgca gttatacttc acataccaat
 721 gaaatccata aaggaaagag ttaccaactg ctggttgttg agaacactgt tgaagtggct
 781 cagttcatta ataacaatcc agaattttta caacttgctg agccattctg gaaggagctt
 841 tcccacctcc cctctctgta tgactacagt gcctaccgaa gattaatcga ccagtacggg
 901 acacattatc tgcaatctgg gtcgttagga ggagaataca gagttctatt ttatgtggac
 961 tcagaaaaat taaaacaaaa tgattttaat tcagtcgaag aaaagaaatg taaatcctca
1021 ggttggcatt ttgtcgttaa attttcaagt catggatgca aggaactgga aaacgcttta
1081 aaagctgctt caggaaccca gaacaatgta ttgcgaggag aaccgttcat cagaggggga
1141 ggtgcaggct tcatatctgg ccttagttac ctagagctgg acaatcctgc tggaaacaaa
1201 aggcgatatt ctgcctgggc agaatctgtg actaatcttc ctcaagtcat aaaacaaaag
1261 ctgacacctt tatatgagct ggtaaaggaa gtaccttgtg cctctgtgaa aaaactatac
1321 ctgaaatggg ctcttgaaga gtatctggat gaatttgacc cctgtcattg ccggccttgt
1381 caaaatggtg gtttggctac tgttgagggg acccattgtc tgtgccattg caaaccgtac
1441 acatttggtg cggcgtgtga gcaaggagtc ctcgtaggga atcaagcagg aggggttgat
1501 ggaggttgga gttgctggtc ctcttggagc ccctgtgtcc aagggaagaa aacaagaagc
1561 cgtgaatgca ataacccacc tcccagtggg ggtgggagat cctgcgttgg agaaacgaca
1621 gaaagcacac aatgcgaaga tgaggagctg gagcacttga ggttgcttga accacattgc
1681 tttcctttgt ctttggttcc aacagaattc tgtccatcac ctcctgcctt gaaagatgga
1741 tttgttcaag atgaaggtcc aatgtttcct gtggggaaaa atgtagtgta cacttgcaat
1801 gaaggatact ctcttattgg aaacccagtg gccagatgtg gagaagattt acggtggctt
1861 gttggggaaa tgcattgtca gaaaattgcc tgtgttctac ctgtactgat ggatggcata
1921 cagagtcacc cccaaaaacc tttctacaca gttggtgaga aggtgactgt ttcctgttca
1981 ggtggcatgt ccttagaagg tccttcagca tttctctgtg gctccagcct taagtggagt
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2101 aaatgtcagc gctgggagaa actgcagaat tcaagatgtg tttgtaaaat gccctacgaa
2161 tgtggacctt ccttggatgt atgtgctcaa gatgagagaa gcaaaaggat actgcctctg
2221 acagtttgca agatgcatgt tctccactgt cagggtagaa attacaccct tactggtagg
2281 gacagctgta ctctgcctgc ctcagctgag aaagcttgtg gtgcctgccc actgtgggga
2341 aaatgtgatg ctgagagcag caaatgtgtc tgccgagaag catcggagtg cgaggaagaa
2401 gggtttagca tttgtgtgga agtgaacggc aaggagcaga cgatgtctga gtgtgaggcg
2461 ggcgctctga gatgcagagg gcagagcatc tctgtcacca gcataaggcc ttgtgctgcg
2521 gaaacccagt aggctcctgg aggccatggt cagcttgctt ggaatccagc aggcagctgg
2581 ggctgagtga aaacatctgc acaactgggc actggacagc ttttccttct tctccagtgt
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2761 ggctggctgc gtgttcttga aataggtgtt accttctctg ggccttggtt ttttaaaatc
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2941 agaaagaacg tgatctcctg attagagagg gtggttttcc tcaatggaac caaatataaa
3001 gaggacttga acaaaaatga cagatacaaa ctatttctat cctgagtagt aatctcacac
3061 ttcatcctat agagtcaacc accacagata ggaattcctt attcttttt taatttttt
3121 aagacagagt ctcactttgt tgcccaggct ggagcgcagt ggggtgatct catctccctg
3181 caacctccgc ctcctgggtt gaagcgattc ttgtgcctca gcttcccaag cagctgggat
3241 tacaggtgcc cgccaccacg cccagctaat ttttgcattt ttagtagaga tgggtttcac
3301 catgttggcc atgctcgtct ccaactcctg acctcaggta atccgtctgc cttggcctcc
3361 caaatgctgg gattacagac atgaaccacc acgcctggct ggaatactta ctcttgtcgg
3421 gagattgaac cactaaaatg ttagagcaga attcattatg ctgtggtcac aggggtgtct
3481 tgtctgagaa caaatacaat tcagtcttct ctttggggtt ttagtatgtg tcaaacatag
3541 gactggaagt ttgcccctgt tctttttttct tttgaaagaa catcagttca tgcctgaggc
```

Figure 10 (cont.)

```
3601 atgagtgact gtgcatttga gatagttttc cctattctgt ggatacagtc ccagagtttt
3661 cagggagtac acaggtagat tagtttgaag cattgacctt ttatttattc cttatttctc
3721 tttcatcaaa acaaaacagc agctgtggga ggagaaatga gagggcttaa atgaaattta
3781 aaataagcta tattatacaa atactatctc tgtattgttc tgaccctggt aaatatattt
3841 caaaacttca gatgacaagg attagaacac tcattaagat gctattcttc

SEQ ID NO:66
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  61 tctctgccgc ccttctgtgc ctgctgctca tagcagccac cttcattccc caagggctcg
 121 ctcagccaga tgcaatcaat gccccagtca cctgctgtta taacttcacc aataggaaga
 181 tctcagtgca gaggctcgcg agctatagaa gaatcaccag cagcaagtgt cccaaagaag
 241 ctgtgatctt caagaccatt gtggccaagg agatctgtgc tgaccccaag cagaagtggg
 301 ttcaggattc catggaccac ctggacaagc aaacccaaac tccgaagact tgaacactca
 361 ctccacaacc caagaatctg cagctaactt attttccccc agctttcccc agacaccctg
 421 ttttatttta ttataatgaa ttttgtttgt tgatgtgaaa cattatgcct taagtaatgt
 481 taattcttat ttaagttatt gatgttttaa gtttatcttt catggtacta gtgtttttta
 541 gatacagaga cttggggaaa ttgcttttcc tcttgaacca cagttctacc cctgggatgt
 601 tttgagggtc tttgcaagaa tcattaatac aaagaatttt ttttaacatt ccaatgcatt
 661 gctaaaatat tattgtggaa atgaatattt tgtaactatt acaccaaata aatatatttt
 721 tgtac

SEQ ID NO:67
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 121 gttggccaac ccaaaaacag aactggagat gtctataagt gtccagttgg gagaggtgaa
 181 tcattacctt gcgtaaagtt ggatctacca gttaatacat caattcccaa tgtcacagaa
 241 gtaaaggaga acatgacatt tggatcaact ttagtcacca acccaaatgg aggatttctg
 301 gcttgtgggc ccttatatgc ctatagatgt ggacatttgc attacacaac tggaatctgt
 361 tctgacgtca gccccacatt tcaagtcgtg aattccattg cccctgtaca agaatgcagc
 421 actcaactgg acatagtcat agtgctggat ggttccaaca gtatttaccc atgggacagt
 481 gttacagctt ttttaaatga ccttcttgaa agaatggata ttggtcctaa acagacacag
 541 gttggaattg tacagtatgg agaaaacgtg acccatgagt tcaacctcaa taagtattct
 601 tccaccgaag aggtacttgt tgcagcaaag aaaatagtcc agagaggtgg ccgccagact
 661 atgacagctc ttggaataga cacagcaaga aaggaggcat tcacggaagc ccggggtgcc
 721 cgaagaggag ttaaaaaagt catggttatt gtgacagatg gagagtctca tgacaatcat
 781 cgactgaaga aggtcatcca agactgtgaa gatgaaaaca ttcaacggtt ttccatagct
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1741 gtggcaagac tataaggaaa gagtatgcac aacgtattcc atcaggtggg gatggtaaga
1801 cactgaaatt ttttggccag tctatccacg gagaaatgga tttaaatggt gacggtctga
1861 cagatgtgac tattgggggc cttggtggtg ctgccctctt ctggtcccga gatgtggccg
```

Figure 10 (cont.)

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2041 agtctaaaga agacacgatt tatgaagctg atttgcagta ccgtgtcacc ctagattcac
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3001 ctgcagaccc catatctttg aggatccttt cagtatcaac tctggaaaga aaatgactac
3061 atcaactgac catctcaaac gaggcacaat tctggactgc aatacatgta aatttgctac
3121 catcacatgt aatctcactt cttctgacat cagccaagtc aatgtttcgc ttatcttgtg
3181 gaaaccaact tttataaaat catatttttc cagcttaaat cttactataa ggggagaact
3241 tcggagtgaa aatgcatctc tggttttaag tagcagcaat caaaaaagag agcttgctat
3301 tcaaatatcc aaagatgggc taccgggcag agtgccatta tgggtcatcc tgctgagtgc
3361 ttttgccgga ttgttgctgt taatgctgct catttagca ctgtggaaga ttggattctt
3421 caaaagacca ctgaaaaaga aaatggagaa a
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SEQ ID NO:68

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 121 gattcaaacc atttgccaaa aatgagtcta agtgcattta ctctcttcct ggcattgatt
 181 ggtggtacca gtggccagta ctatgattat gattttcccc tatcaattta tgggcaatca
 241 tcaccaaact gtgcaccaga atgtaactgc cctgaaagct acccaagtgc catgtactgt
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 781 aacaataaga tcagcaacat ccctgatgag tatttcaagc gttttaatgc attgcagtat
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1021 ttctgcaaga tcctggggcc attatcctac tccaagatca agcatttgcg tttggatggc
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1261 atctgaggga aatgttttgt aaacatttat ttttttaaa gaaaagatga aaggcaggcc
1321 tatttcatca caagaacaca cacatataca cgaatagaca tcaaactcaa tgctttattt
1381 gtaaatttag tgtttttta tttctactgt caaatgatgt gcaaaacctt ttactggttg
1441 catggaaatc agccaagttt tataatcctt aaatcttaat gttcctcaaa gcttggatta
1501 aatacatatg gatgttactc tcttgcacca aattatcttg atacattcaa atttgtctgg
```

Figure 10 (cont.)

```
1561 ttaaaaaata ggtggtagat attgaggcca agaatattgc aaaatacatg aagcttcatg
1621 cacttaaaga agtattttta gaataagaat ttgcatactt acctagtgaa acttttctag
1681 aattattttt cactctaagt catgtatgtt tctctttgat tatttgcatg ttatgtttaa
1741 taagctacta gcaaaataaa acatagcaaa tg

SEQ ID NO:69
   1 tggacagagg agcagtaaca atccccactc tccaattgtg gaagagttcc aagtcccata
  61 caacaaactc caggtgatct ttaagtcaga cttttccaat gaagagcgtt ttacggggtt
 121 tgctgcatac tatgttgcca cagacataaa tgaatgcaca gattttgtag atgtcccttg
 181 tagccacttc tgcaacaatt tcattggtgg ttacttctgc tcctgccccc cggaatattt
 241 cctccatgat gacatgaaga attgcggagt taattgcagt ggggatgtat tcactgcact
 301 gattggggag attgcaagtc ccaattatcc caaaccatat ccagagaact caaggtgtga
 361 ataccagatc cggttggaga aagggttcca agtggtggtg accttgcgga gagaagattt
 421 tgatgtggaa gcagctgact cagcgggaaa ctgccttgac agtttagttt ttgttgcagg
 481 agatcggcaa tttggtcctt actgtggtca tggattccct gggcctctaa atattgaaac
 541 caagagtaat gctcttgata tcatcttcca aactgatcta acagggcaaa aaaagggctg
 601 gaaacttcgc tatcatggag atccaatgcc ctgccctaag gaagacactc ccaattctgt
 661 ttgggagcct gcgaaggcaa aatatgtctt tagagatgtg gtgcagataa cctgtctgga
 721 tgggtttgaa gttgtggagg gacgtgttgg tgcaacatct ttctattcga cttgtcaaag
 781 caatggaaag tggagtaatt ccaaactgaa atgtcaacct gtggactgtg gcattcctga
 841 atccattgag aatggtaaag ttgaagaccc agagagcact ttgtttggtt ctgtcatccg
 901 ctacacttgt gaggagccat attactacat ggaaaatgga ggaggtgggg agtatcactg
 961 tgctggtaac gggagctggg tgaatgaggt gctgggcccg gagctgccga aatgtgttcc
1021 aggtctgtgg agtccccaga gaacccttg aagaaaaaca gaggataatt ggaggatccg
1081 atgcagatat taaaaacttc ccctggcaag tcttctttga caacccatgg gctggtggag
1141 cgctcattaa tgagtactgg gtgctgacgg ctgctcatgt tgtggaggga aacagggagc
1201 caacaatgta tgttgggtcc acctcagtgc agacctcacg gctggcaaaa tccaagatgc
1261 tcactcctga gcatgtgttt attcatccgg gatggaagct gctggaagtc ccagaaggac
1321 gaaccaattt tgataatgac attgcactgg tgcggctgaa agacccagtg aaaatgggac
1381 ccaccgtctc tcccatctgc ctaccaggca cctcttccga ctacaacctc atggatgggg
1441 acctgggact gatctcaggc tggggccgaa cagagaagag agatcgtgct gttcgcctca
1501 aggcggcaag gttacctgta gctcctttaa gaaaatgcaa agaagtgaaa gtggagaaac
1561 ccacagcaga tgcagaggcc tatgttttca ctcctaacat gatctgtgct ggaggagaga
1621 agggcatgga tagctgtaaa ggggacagtg gtggggcctt tgctgtacag gatcccaatg
1681 acaagaccaa attctacgca gctggcctgg tgtcctgggg gccccagtgt gggacctatg
1741 ggctctacac acgggtaaag aactatgttg actggataat gaagactatg caggaaaata
1801 gcaccccccg tgaggactaa tccagataca tcccaccagc ctctccaagg gtggtgacca
1861 atgcattacc ttctgttcct tatgatattc tcattatttc atcatgactg aaagaagaca
1921 cgagcgaatg atttaaatag aacttgattg ttgagacgcc ttgctagagg tagagtttga
1981 tcatagaatt gtgctggtca tacatttgtg gtctgactcc ttggggtcct ttccccggag
2041 tacctattgt agataacact atgggtgggg cactcctttc ttgcactatt ccacagggat
2101 accttaattc tttgtttcct ctttacctgt tcaaaattcc atttacttga tcattctcag
2161 tatccactgt ctatgtacaa taaaggatgt ttataagc

SEQ ID NO:70
   1 aaactctgat ctggggagga accaggacta catagatcaa ggcagttttc ttctttgaga
  61 aactatccca gatatcatca tagagtcttc tgctcttcct caactaccaa agaaaaacat
 121 cagcgaagca gcaggccatg caccccccaa aaactccatc tggggctctt catagaaaaa
 181 ggaaaatggc agcctggccc ttctccaggc tgtggaaagt ctctgatcca attctcttcc
 241 aaatgacctt gatcgctgct ctgttgcctg ctgttcttgg caattgtggt cctccaccca
 301 ctttatcatt tgctgccccg atggatatta cgttgactga gacacgcttc aaaactggaa
 361 ctactctgaa atacacctgc ctccctggct acgtcagatc ccattcaact cagacgctta
 421 cctgtaattc tgatggcgaa tgggtgtata acaccttctg tatctacaaa cgatgcagac
 481 acccaggaga gttacgtaat gggcaagtag agattaagac agatttatct tttggatcac
```

Figure 10 (cont.)

```
 541 aaatagaatt cagctgttca gaaggatttt tcttaattgg ctcaaccact agtcgttgtg
 601 aagtccaaga tagaggagtt ggctggagtc atcctctccc acaatgtgaa attgtcaagt
 661 gtaagcctcc tccagacatc aggaatggaa ggcacagcgg tgaagaaaat ttctacgcat
 721 acggcttttc tgtcacctac agctgtgacc cccgcttctc actcttgggc catgcctcca
 781 tttcttgcac tgtggagaat gaaacaatag gtgtttggag accaagccct cctacctgtg
 841 aaaaaatcac ctgtcgcaag ccagatgttt cacatgggga aatggtctct ggatttggac
 901 ccatctataa ttacaaagac actattgtgt ttaagtgcca aaaaggtttt gttctcagag
 961 gcagcagtgt aattcattgt gatgctgata gcaaatggaa tccttctcct cctgcttgtg
1021 agcccaatag ttgtattaat ttaccagaca ttccacatgc ttcctgggaa acatatccta
1081 ggccgacaaa agaggatgtg tatgttgttg ggactgtgtt aaggtaccgc tgtcatcctg
1141 gctacaaacc cactacagat gagcctacga ctgtgatttg tcagaaaaat ttgagatgga
1201 ccccatacca aggatgtgag gcgttatgtt gccctgaacc aaagctaaat aatggtgaaa
1261 tcactcaaca caggaaaagt cgtcctgcca atcactgtgt ttatttctat ggagatgaga
1321 tttcattttc atgtcatgag accagtaggt tttcagctat atgccaagga gatggcacgt
1381 ggagtccccg aacaccatca tgtggagaca tttgcaattt tcctcctaaa attgcccatg
1441 ggcattataa acaatctagt tcatacagct ttttcaaaga agagattata tatgaatgtg
1501 ataaaggcta cattctggtc ggacaggcga aactctcctg cagttattca cactggtcag
1561 ctccagcccc tcaatgtaaa gctctgtgtc ggaaaccaga attagtgaat ggaaggttgt
1621 ctgtggataa ggatcagtat gttgagcctg aaaatgtcac catccaatgt gattctggct
1681 atggtgtggt tggtccccaa agtatcactt gctctgggaa cagaacctgg tacccagagg
1741 tgcccaagtg tgagtgggag acccccgaag gctgtgaaca agtgctcaca ggcaaaagac
1801 tcatgcagtg tctcccaaac ccagaggatg tgaaaatggc cctggaggta tataagctgt
1861 ctctggaaat tgaacaactg gaactacaga gagacagcgc aagacaatcc actttggata
1921 aagaactata atttttctca aaagaaggag gaaaaggtgt cttgctggct tgcctcttgc
1981 aattcaatac agatcagttt agcaaatcta ctgtcaattt ggcagtgata ttcatcataa
2041 taaatatcta gaaatgataa tttgctaaag tttagtgctt tgagattgtg aaattattaa
2101 tcatcctctg tgtggctcat gtttttgctt ttcaacacac aaagcacaaa tttttttttcg
2161 attaaaaatg tatgtat
```

SEQ Id NO:71

```
   1 gccctgctgg ccctgctggt gctcccnnnn nnnnnnnnnn nnnnnnnnnn nnnggtcctc
  61 aaggcccacg tggtgacaaa ggtgaaacag gtgaacgtgg agctgctggc atcaaaggac
 121 atcgaggatt ccctggtaat ccaggtgccc caggttctcc agggccctgc tggtcagcag
 181 ggtgcaatcg gcagtccagg acctgcaggc cccagaggac ctgttggacc cagtggacct
 241 cctggcaaag atggaaccag tggacatcca ggtcccattg gaccaccagg gcctcgaggt
 301 aacagaggtg aaagaggatc tgagggctcc ccaggccacc cagggcaacc aggccctcct
 361 ggacctcctg gtgcccctgg tccttgc
```

SEQ Id NO:72

```
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  61 ccggcgaggg ccccggcagg cccggtccct gctcgggggc gcgctgagac ggcgggtgag
 121 ctccacgaga gcgccgtcgc cacttcgggc caactttgcg attcccgaca gttaagcaat
 181 ggggagacat ttggctttgc tcctgcttct gctccttctc ttccaacatt ttggagacag
 241 tgatggcagc caacgacttg aacagactcc tctgcagttt acacacctcg agtacaacgt
 301 caccgtgcag gagaactctg cagctaagac ttatgtgggg catcctgtca agatgggtgt
 361 ttacattaca catccagcgt gggaagtaag gtacaaaatt gtttccggag acagtgaaaa
 421 cctgttcaaa gctgaagagt acattctcgg agacttttgc tttctaagaa taaggaccaa
 481 aggaggaaat acagctattc ttaatagaga agtgaaggat cactacacat tgatagtgaa
 541 agcacttgaa aaaaatacta atgtggaggc gcgaacaaag gtcagggtgc aggtgctgga
 601 tacaaatgac ttgagaccgt tattctcacc cacctcatac agcgtttctt tacctgaaaa
 661 cacagctata aggaccagta tcgcaagagt cagcgccacg gatgcagaca taggaaccaa
 721 cggggaattt tactacagtt ttaaagatcg aacagatatg tttgctattc acccaaccag
 781 tggtgtgata gtgttaactg gtagacttga ttacctagag accaagctct atgagatgga
 841 aatcctcgct gcggaccgtg gcatgaagtt gtatgggagc agtggcatca gcagcatggc
```

Figure 10 (cont.)

```
 901 caagctaacg gtgcacatcg aacaggccaa tgaatgtgct ccggtgataa cagcagtgac
 961 attgtcacca tcagaactgg acagggaccc agcatatgca attgtgacag tggatgactg
1021 cgatcagggt gccaatggtg acatagcatc tttaagcatc gtggcaggtg accttctcca
1081 gcagtttaga acagtgaggt cctttccagg gagtaaggag tataaagtca aagccatcgg
1141 tggcattgat tgggacagtc atcctttcgg ctacaatctc acactacagg ctaaagataa
1201 aggaactccg ccccagttct cttctgttaa agtcattcac gtgacttctc cacagttcaa
1261 agccgggcca gtcaagtttg aaaaggatgt ttacagagca gaaataagtg aatttgctcc
1321 tcccaacaca cctgtggtca tggtaaaggc cattcctgct tattcccatt tgaggtatgt
1381 ttttaaaagt acacctggaa aagctaaatt cagtttaaat tacaacactg gtctcatttc
1441 tattttagaa ccagttaaaa gacagcaggc agcccatttt gaacttgaag taacaacaag
1501 tgacagaaaa gcgtccacca aggtcttggt gaaagtctta ggtgcaaata gcaatccccc
1561 tgaatttacc cagacagcgt acaaagctgc ttttgatgag aacgtgccca ttggtactac
1621 tgtcatgagc ctgagtgccg tagaccctga tgagggtgag aacgggtacg tgacatacag
1681 tatcgcaaat ttaaatcatg tgccgtttgc gattgaccat ttcactggtg ccgtgagtac
1741 gtcagaaaac ctggactacg aactgatgcc tcgggtttat actctgagga ttcgtgcatc
1801 agactggggc ttgccgtacc gccgggaagt cgaagtcctt gctacaatta ctctcaataa
1861 cttgaatgac aacacacctt tgtttgagaa aataaattgt gaagggacaa ttcccagaga
1921 tctaggcgtg ggagagcaaa taaccactgt ttctgctatt gatgcagatg aacttcagtt
1981 ggtacagtat cagattgaag ctggaaatga actggatttc tttagtttaa accccaactc
2041 gggggtattg tcattaaagc gatcgctaat ggatggctta ggtgcaaagg tgtctttcac
2101 agtctgagaa tcacagctac agatggagaa aattttgcca caccattata tatcaacata
2161 acagtggctg ccagtcacaa gctggtaaac ttgcagtgtg aagagactgg tgttgccaaa
2221 atgctggcag agaagctcct gcaggcaaat aaattacaca accagggaga ggtggaggat
2281 attttcttcg attctcactc tgtcaatgct cacataccgc agtttagaag cactcttccg
2341 actggtattc aggtaaagga aaaccagcct gtgggttcca gtgtaatttt catgaactcc
2401 actgaccttg acactggctt caatggaaaa ctggtctatg ctgtttctgg aggaaatgag
2461 gatagttgct tcatgattga tatggaaaca ggaatgctga aaattttatc tcctcttgac
2521 cgtgaaacaa cagacaaata caccctgaat attaccgtct atgaccttgg gataccccag
2581 aaggctgcgt ggcgtcttct acatgtcgtg gttgtcgatg ccaatgataa tccacccgag
2641 tttttacagg agagctattt tgtggaagtg agtgaagaca aggaggtaca tagtgaaatc
2701 atccaggttg aagccacaga taaagacctg gggcccaacg gacacgtgac gtactcaatt
2761 gttacagaca cagacacatt ttcaattgac agcgtgacgg gtgttgttaa catcgcacgc
2821 cctctggatc gagagctgca gcatgagcac tccttaaaga ttgaggccag ggaccaagcc
2881 agagaagagc ctcagctgtt ctccactgtc gttgtgaaag tatcactaga agatgttaat
2941 gacaacccac ctacatttat tccacctaat tatcgtgtga aagtccgaga ggatcttcca
3001 gaaggaaccg tcatcatgtg gttagaagcc cacgatcctg atttaggtca gtctggtcag
3061 gtcagcacac agccttctgg accacggaga aggaaacttc gatgtggata aactcagtgg
3121 agcagttagg atcgtccagc agttggactt tgagaagaag caagtgtata atctcactgt
3181 gagggccaaa gacaagggaa agccagtttc tctgtcttct acttgctatg ttgaagttga
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3301 gacagtgaaa gaagatgcac ctgttggttc attggtaatg acggtgtcgg ctcatgatga
3361 ggacgccaga agagatgggg agatccgata ctccattaga gatggctctg gcgttggtgt
3421 tttcaaaata ggtgaagaga caggtgtcat agagacgtca gatcgactgg accgtgaatc
3481 gacctcccat tattggctaa cagtctttgc aaccgatcag ggtgtcgtgc ctctttcatc
3541 gttcatagag atctacatag aggttgagga tgtcaatgac aatgcaccac agacatcaga
3601 gcctgtttat tacccagaaa tcatggaaaa ttctcctaaa gatgtatctg tggtccagat
3661 cgaggcattt gatccagatt cgagctctaa tgacaagctc atgtacaaaa ttacaagtgg
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3781 gaagctagac cgagaacagc aagatgaaca catattagag gttactgtga cagacaatgg
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3901 caaacctcag tttctgcaaa agttctacaa aatcagactc cctgagcggg aaaagccaga
3961 ccgagaaaga aatgccagac gggagccgct ctatcgcgtc atagccaccg acaaggatga
4021 gggccccaat gcagaaatct cctacagcat cgaagacggg aatgagcatg gcaaatttttt
4081 catcgaaccg aaaactggag tggtttcgtc caagaggttt tcagcagctg gagaatatga
```

Figure 10 (cont.)

```
4141 tattctttca attaaggcag ttgacaatgg tcgccctcaa aagtcatcaa ccaccagact
4201 ccatattgaa tggatctcca agcccaaacc gtccctggag cccatttcat ttgaagaatc
4261 atttttacc tttactgtga tggaaagtga ccccgttgct cacatgattg gagtaatatc
4321 tgtggagcct cctggcatac ccctttggtt tgacatcact ggtggcaact acgacagtca
4381 cttcgatgtg gacaagggaa ctggaaccat cattgttgcc aaacctcttg atgcagaaca
4441 gaagtcaaac tacaacctca cagtcgaggc tacagatgga accaccacta tcctcactca
4501 ggtattcatc aaagtaatag acacaaatga ccatcgtcct cagttttcta catcaaagta
4561 tgaagttgtt attcctgaag atacagcgcc agaaacagaa attttgcaaa tcagtgctgt
4621 ggatcaggat gagaaaaaca aactaatcta cactctgcag agcagtagag atccactgag
4681 tctcaagaaa tttcgtcttg atcctgcaac cggctctctc tatacttctg agaaactgga
4741 tcatgaagct gttcaccagc acaccctcac ggtcatggta cgagatcaag atgtgcctgt
4801 aaaacgcaac tttgcaagga ttgtggtcaa tgtcagcgac acgaatgacc acgccccgtg
4861 gttcaccgct tcctcctaca aagggcgggt ttatgaatcg gcagccgttg gctcagttgt
4921 gttgcaggtg acggctctgg acaaggacaa agggaaaaat gctgaagtgc tgtactcgat
4981 cgagtcagnn nnnnnnngaa atattggaaa ttcttttatg attgatcctg tcttgggctc
5041 tattaaaact gccaaagaat tagatcgaag taaccaagcg gagtatgatt taatggtaaa
5101 agctacagat aagggcagtc caccaatgag tgaaataact tctgtgcgta tctttgtcac
5161 aattgctgac aacgcctctc cgaagtttac atcaaagaa tattctgttg aacttagtga
5221 aactgtcagc attgggagtt tcgttgggat ggttacagcc catagtcaat catcagtggt
5281 gtatgaaata aaagatggaa atacaggtga tgcttttgat attaatccac attctggaac
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5461 gaatgacaac gcgccagttt ttatgcaggc agaatataca ggactcatta gtgaatcagc
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5761 tgctgagtat gcagcgaatg taacagtaca tgtaattgac attaatgact gccccctgt
5821 gtttgccaag ccattatatg aagcatctct tttgttacca acatacaaag gagtaaaagt
5881 catcacagta aatgctacag atgctgattc aagtgcattc tcacagttga tttactccat
5941 caccgaaggc aacatcgggg agaagttttc tatggactac aagactggtg ctctcactgt
6001 ccaaaacaca actcagttaa gaagccgcta cgagctaacc gttagagctt ccgatggcag
6061 atttgccggc cttacctctg tcaaaattaa tgtgaaagaa agcaaagaaa gtcacctaaa
6121 gtttacccag gatgtctact ctgcggtagt gaaagagaat tccaccgagg ccgaaacatt
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6481 tgtcattcgc tatgtcactg ctgtagacag agacagtggc agaaacgggg aagtgcatta
6541 ctacctcaag gaacatcatg aacactttca aattggaccc ttgggtgaaa tttcactgaa
6601 aaagcaattt gagcttgaca ccttaaataa agaatatctt gttacagtgg ttgcaaaaga
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6901 catagctcct ctggactttg aggcccaccc ggcatataag ctgagcatac gcgcaactga
6961 ctccttgacg ggcgctcatg ctgaagtatt tgtggacatc atagtagacg acatcaatga
7021 taaccctcct gtgtttgctc agcagtctta tgcggtgacc ctgtctgagg catctgtaat
7081 tggaacgtct gttgttcaag ttagagccac cgattctgat tcagaaccaa atagaggaat
7141 ctcataccag atgtttggga atcacagcaa gagtcatgat cattttcatg tagacagcag
7201 cactggcctc atctcactac tcagaaccct ggattacgag cagtcccggc agcacacgat
7261 ttttgtgagg gcagttgatg gtggtatgcc cacgctgagc agtgatgtga ttgtcacggt
7321 ggacgttacc gacctcaatg ataatccacc actctttgaa caacagattt atgaagccag
```

Figure 10 (cont.)

```
 7381 aattagcgag cacgcccctc atgggcattt cgtgacctgt gtaaaagcct atgatgcaga
 7441 cagttcagac atagacaagt tgcagtattc cattctgtct ggcaatgatc ataaacattt
 7501 tgtcattgac agtgcaacag ggattatcac cctctcaaac ctgcaccggc acgccctgaa
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 7801 cagattttac ataaatgaga gaggacagat atttactttg gaaaaacttg atcgagaaac
 7861 cccggcggag aaagtgatct cagtccgttt aatggctaag gatgctggag gaaaagttgc
 7921 tttctgcacc gtgaatgtca tccttacaga tgacaatgac aatgcaccac aatttcgagc
 7981 aaccaaatac gaagtgaata tcgggtccag tgctgctaaa gggacttcag tcgttaaagt
 8041 tcttgcaagt gatgccgatg agggctccaa tgccgacatc acctatgcca ttgaagcaga
 8101 ctctgaaagt gtaaaagaga atttggaaat taacaaactg tccggcgtaa tcactacaaa
 8161 ggagagcctc attggcttgg aaaatgaatt cttcactttc tttgttagag ctgtggataa
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Figure 10 (cont.)

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10741 agataatgga aagcctcagt tgtcatcttt gacatacatt gacattaggg taattgagga
10801 gagcatctat ccgcctgcga ttttgcccct ggagattttc atcacctctt ctggagaaga
10861 atactcaggt ggcgtcattg ggaagatcca tgccacagac caggacgtgt atgatactct
10921 aacctacagt ctcgaccctc agatggacaa cctgttctct gtttccagca caggggcaa
10981 gctgatagca cacaaaaagc tagacatagg gcaatacctt ctcaatgtca gcgtaacaga
11041 tgggaagttc acgacggtgg ccgacatcac agtgcatatc agacaagtca cacaggagat
11101 gttgaaccac accatcgcga tccgctttgc caacctcact ccggaagaat tcgttggtga
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11221 acagattgtt agtttgcagt cctctgaacc tcacccacat ctggacgtct tacttttgt
11281 agagaaacca ggtagtgctc agatctcaac aaaacaactt ctgcacaaga ttaactcttc
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11881 nnnnnnnnnn nnncattcag gtcaatgatg ggcagtggca cgcagtggcc ctggaagtga
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12061 agggaacaag gcatggaaga agtcctcaag ttggtaatgg tttcaggggt tgtatggact
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12181 tcgaagagtc ggtggatgta tctccaggct gcttcctgac ggccacggaa gactgcgcca
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12301 aatgcagtgc cttgtacata gggacccact gtgagataag cgtcaatccg tgttcctcca
12361 agccatgcct ctatgggggc acgtgtgttg tcgacaacgg aggctttgtt tgccagtgta
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13561 ttgaatccat ccaccctcct agagacatgc ctgccgcggg tagcttgggt tcttcatcaa
13621 gaaaccggca gaggttcaac ttgaatcagt atttgcccaa tttttatccc ctcgatatgt
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13741 acccgccagg gtatcaaaga cacttcgagg cgcccgctgt cgagagcatg cccatgtctg
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Figure 10 (cont.)

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14161 aacgtctttt gggatttaca tctgtctgtg ttaaaataat caaacgaaaa atcagtcctg
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14641 acagttttta tagtgtctgt atatttgtga tgcaatggtc ttgtaaaggt ttttaatgaa
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SEQ Id NO:73

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  181 gcaagtcatc agactgcata aaatcagctg ctcgactgat ccaaaacatg gatgccacca
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  601 aaaaagtcct tattaatttg tttgttggca ctgatgataa gaattctgtg aatcatgtaa
  661 ttcatattga ccaacctcga cttggcctcc cttctagaga ttactatgaa tgcactggaa
  721 tctataaaga ggcttgtaca gcatatgtgg attttatgat ttctgtggcc agattgattc
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  901 tgcttctgta taacaagatg acattggccc agatccaaaa taacttttca ctagagatca
  961 atgggaagcc attcagctgg ttgaatttca caaatgaaat catgtcaact gtgaatatta
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Figure 10 (cont.)

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2821 tcattctgtg atcatttatt ttaagcactc ttaaagcaaa aaatgaatgt ctaaaattgt
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Figure 10 (cont.)

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  61 tgcctcacct acccccaacc tcccagaggg agcagctatt taaggggagc aggagtgcag
 121 aacaaacaag acggcctggg gatacaactc tggagtcctc tgagagagcc accaaggagg
 181 agcaggggag cgacggccgg ggcagaagtt gagaccaccc agcagaggag ctaggccagt
 241 ccatctgcat ttgtcaccca agaactctta ccatgaagac cctcctactg ttggcagtga
 301 tcatgatctt tggcctactg caggcccatg ggaatttggt gaatttccac agaatgatca
 361 agttgacgac aggaaaggaa gccgcactca gttatggctt ctacggctgc cactgtggcg
 421 tgggtggcag aggatccccc aaggatgcaa cggatcgctg ctgtgtcact catgactgtt
 481 gctacaaacg tctggagaaa cgtggatgtg gcaccaaatt tctgagctac aagtttagca
 541 actcggggag cagaatcacc tgtgcaaaac aggactcctg cagaagtcaa ctgtgtgagt
 601 gtgataaggc tgctgccacc tgttttgcta gaaacaagac gacctacaat aaaaagtacc
 661 agtactattc caataaacac tgcagaggga gcacccctcg ttgctgagtc ccctcttccc
 721 tggaaacctt ccacccagtg ctgaatttcc ctctctcata ccctccctcc ctaccctaac
 781 caagttcctt ggccatgcag aaagcatccc tcacccatcc tagaggccag gcaggagccc
 841 ttctataccc acccagaatg agacatccag cagatttcca gccttctact gctctcctcc
 901 acctcaactc cgtgcttaac caaagaagct gtactccggg gggtctcttc tgaataaagc
 961 aattagc

SEQ ID NO:76
   1 gctccatcaa gtatgatggt gaaggatgaa tatgtgcatg actttgaggg acagccatcg
  61 ttgtccactg aaggacattc aattcaaacc atccagcatc caccaagtaa tcgtgcatcg
 121 acagagacat acagcacccc agctctgtta gccccatctg agtctaatgc taccagcact
```

Figure 10 (cont.)

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 181 gccaactttc ccaacattcc tgtggcttcc acaagtcagc ctgccagtat actgggggc
 241 agccatagtg aaggactgtt gcagatagca tcagggcctc agccaggaca gcagcagaat
 301 ggatttactg gtcagccagc tacttaccat cataacagca ctaccacctg gactggaagt
 361 aggactgcac catacacacc taatttgcct caccaccaaa acggccatct tcagcaccac
 421 ccgcctatgc cgccccatcc cggacattac tggcctgttc acaatgagct tgcattccag
 481 cctcccattt ccaatcatcc tgctcctgag tattggtgtt ccattgctta ctttgaaatg
 541 gatgttcagg taggagagac atttaaggtt ccttcaagct gccctattgt tactgttgat
 601 ggatacgtgg accettctgg aggagatcgc ttttgtttgg gtcaactctc caatgtccac
 661 aggacagaag ccattgagag agcaaggttg cacataggca aaggtgtgca gttggaatgt
 721 aaaggtgaag gtgatgtttg ggtcaggtgc cttagtgacc acgcggtctt tgtacagagt
 781 tactacttag acagagaagc tgggcgtgca cctggagatg ctgttcataa gatctaccca
 841 agtgcatata taaaggtctt tgatttgcgt cagtgtcatc gacagatgca gcagcaggcg
 901 gctactgcac aagctgcagc agctgcccag gcagcagccg tggcaggaaa catccctggc
 961 ccaggatcag taggtggaat agctccagct atcagtctgt cagctgctgc tggaattggt
1021 gttgatgacc ttcgtcgctt atgcatactc aggatgagtt ttgtgaaagg ctggggaccg
1081 gattacccaa gacagagcat caaagaaaca ccttgctgga ttgaaattca cttacaccgg
1141 gccctccagc tcctagacga agtacttcat accatgccga ttgcagaccc acaaccttta
1201 gactgaggtc ttttaccgtt ggggcccttа accttatcag gatggtggac tacaaaatac
1261 aatcctgttt ataatctgaa gatatatttc acttttgttc tgctttatct tttcataaag
1321 ggttgaaaat gtgtttgctg ccttgctcct agcagacaga aactggatta aaacaatttt
1381 ttttttcctc ttcagaactt gtcaggcatg gctcagagct tgaagattag gagaaacaca
1441 ttcttattaa ttcttcacct gttatgtatg aaggaatcat tccagtgcta gaaaatttag
1501 ccctttaaaa cgtcttagag ccttttatct gcagaacatc gatatgtata tcattctaca
1561 gaataatcca gtattgctga ttttaaaggc agagaagttc tcaaagttaa ttcacctatg
1621 ttattttgtg tacaagttgt tattgttgaa catacttcaa aaataatgtg ccatgtgggt
1681 gagttaattt taccaagagt aactttactc tgtgtttaaa aagtaagtta ataatgtatt
1741 gtaatctttc atccaaaata ttttttgcaa gttatattag tgaagatggt ttcaattcag
1801 attgtcttgc aacttcagtt ttatttttgc caaggcaaaa aactcttaat ctgtgtgtat
1861 attgagaatc ccttaaaatt accagacaaa aaaatttaaa attacgtttg ttattcctag
1921 tggatgactg ttgatgaagt atacttttcc cctgttaaac agtagttgta ttcttctgta
1981 tttctaggca caaggttggt tgctaagaag cctataagag gaatttcttt tccttcattc
2041 atagggaaag gttttgtatt ttttaaaaca ctaaaagcag cgtcactcta cctaatgtct
2101 cactgttctg caaaggtggc aatgcttaaa ctaaataatg aataaactga atattttgga
2161 aactgctaaa ttctatgtta aatactgtgc agaataatgg aaacattaca gttcataata
2221 ggtagtttgg atatttttgt acttgatttg atgtgacttt ttttggtata atgtttaaat
2281 catgtatgtt atgatattgt ttaaaattca gtttttgtat cttggggcaa gactgcaaac
2341 ttttttatat cttttggtta ttctaagccc tttgccatca atgatcatat caattggcag
2401 tgactttgta tagagaattt aagtagaaaa gttgcagatg tattgactgt accacagaca
2461 caatatgtat gctttttacc tagctggtag cataaataaa actgaatctc aacat
```

SEQ ID NO:77

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   1 gcaggcccgt tggaagtggt tgtgacaacc ccagcaatgt ggagaagcct ggggcttgcc
  61 ctggctctct gtctcctccc atcgggagga acagagagcc aggaccaaag ctccttatgt
 121 aagcaacccc cagcctggag cataagagat caagatccaa tgctaaactc caatggttca
 181 gtgactgtgg ttgctcttct tcaagccagc tgatacctgt gcatactgca ggcatctaaa
 241 ttagaagacc tgcgagtaaa actgaagaaa gaaggatatt ctaatatttc ttatattgtt
 301 gttaatcatc aaggaatctc ttctcgatta aaatacacac atcttaagaa taaggtttca
 361 gagcatattc ctgtttatca acaagaagaa aaccaaacag atgtctggac tcttttaaat
 421 ggaagcaaag atgacttcct catatatgat agatgtggcc gtcttgtata tcatcttggt
 481 ttgccttttt ccttcctaac tttcccatat gtagaagaag ccattaagat tgcttactgt
 541 gaaaagaaat gtggaaactg ctctctcacg actctcaaag atgaagactt ttgtaaacgt
 601 gtatctttgg ctactgtgga taaaacagtt gaaactccat cgcctcatta ccatcatgag
 661 catcatcaca atcatggaca tcagcacctt ggcagcagtg agctttcaga gaatcagcaa
 721 ccaggagcac caaatgctcc tactcatcct gctcctccag gccttcatca ccaccataag
```

Figure 10 (cont.)

```
 781 cacaagggtc agcataggca gggtcaccca gagaaccgag atatgccagc aagtgaagat
 841 ttacaagatt tacaaaagaa gctctgtcga aagagatgta taaatcaatt actctgtaaa
 901 ttgcccacag attcagagtt ggctcctagg agctgatgct gccattgtcg acatctgata
 961 tttgaaaaaa cagggtctgc aatcacctga cagtgtaaag aaaacctccc atctttatgt
1021 agctgacagg gacttcgggc agaggagaac ataactgaat cttgtcagtg acgtttgcct
1081 ccagctgcct gacaaataag tcagcagctt atacccacag aagccagtgc cagttgacgc
1141 tgaaagaatc aggcaaaaaa gtgagaatga ccttcaaact aaatatttaa aataggacat
1201 actccccaat ttagtctaga cacaatttca tttccagcat ttttataaac taccaaatta
1261 gtgaaccaaa aatagaaatt agatttgtgc aaacatggag aaatctactg aattggcttc
1321 cagattttaa attttatgtc atagaaatat tgactcaaac catatttttt atgatggagc
1381 aactgaaagg tgattgcagc ttttggttaa tatgtctttt tttttctttt tccagtgttc
1441 tatttgcttt aatgagaata gaaacgtaaa ctatgaccta ggggtttctg ttggataatt
1501 agcagtttag aatggaggaa gaacaacaaa gacatgcttt ccatttttt ctttacttat
1561 ctctcaaaac aatattactt tgtcttttca atcttctact tttaactaat aaaataagtg
1621 gattttgtat tttaagatcc agaaatactt aacacgtgaa tattttgcta aaaaagcata
1681 tataactatt ttaaatatcc atttatcttt tgtatatcta agactcatcc tgatttttac
1741 tatcacacat gaataaagcc tttgtatctt tctttctcta atgttgtatc atactcttct
1801 aaaacttgag tggctgtctt aaaagatata aggggaaaga taatattgtc tgtctctata
1861 ttgcttagta agtatttcca tagtcaatga tggtttaata ggtaaaccaa accctataaa
1921 cctgacctcc tttatggtta atactattaa gcaagaatgc agtacagaat tggatacagt
1981 acggatttgt ccaaataaat tcaataaaaa ccttaaa
```

SEQ ID NO:78

```
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 121 tgaagctttt gcacgagaac atggactcat cttcatggaa acgtctgcta agactgcttc
 181 caatgtagaa gaggcattta ttaatacagc aaaagaaatt tatgaaaaaa ttcaagaagg
 241 agtctttgac attaataatg aggcaaatgg cattaaaatt ggccctcagc atgctgctac
 301 caatgcaaca catgcaggca atcagggagg acagcaggct gggggcggct gctgttgagt
 361 ctgtttttac tgtctagctg cccaacgggg cctactcact tattctttca ccccctctcc
 421 tcctgctcag ctgagacatg aaactatttg aaatggcttt atgtcacaga agactttaat
 481 ccgtcaaatt cttgtataac tttgaataaa tggttaatgt tcacttaaaa gacagatttt
 541 ggagattgta ttcatatcta tttgcatttg atttctaggt caattgatgt gattattttt
 601 gttaaatgtt gtcttgtgcc cttaactacg aactgaattg tattaaacac tacaaagtca
 661 tcttgagtat tttaaatcgg tttgtgtagt taggtttccc aacatctgtg gttacctaat
 721 gtttaatatt atagaactgt cctcagaaac tttgtcaatt ttcacggcta taaggaaaca
 781 gaaggactct tttaattctg tatttatcat ttactttctg tatatatagt ttaataacct
 841 gcttgggtgt aatttgccaa gcttgaattc tttaatgcat ttgcataaat tctatactgt
 901 ttagagctta aagctacaga agcattgtta ggaattgctt ggacactgaa ttttaaactt
 961 tttgacattg ttaacaagca tgttcatctt ttcttgtcac tagtccaaga aaaatatgct
1021 taatgtatat tacaaaggct ttgtatatgt taacctgttt taatgccaaa agtttgcttt
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1201 gactccaaga agaatgagta tccacattta gatggcacat tatgaggact ttaatctttc
1261 cttaaacaca ataatgtttt ctttttttctt ttattcacat gatttctaag tatatttttc
1321 atgcaggaca gtttttcaac cttgatgtac agtgactgtg taaaattttt ctttcagtgg
1381 caacctctat aatctttaaa atatggtgag catcttgtct gttttgaagg ggatatgaca
1441 ataaatctat cagatggaaa atcctgtt
```

SEQ ID NO:79

```
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  61 gggtgcctcc gagtgtgcgc gcgctctcgc tacccggcgg ggaggggggtg gggggagggc
 121 ccgggaaaag ggggagttgg agccggggtc gaaacgccgc gtgacttgta ggtgagagaa
```

Figure 10 (cont.)

```
 181 cgccgagccg tcgccgcagc ctccgccgcc gagaagccct tgttcccgct gctgggaagg
 241 agagtctgtg ccgacaagat ggcggacggg gagctgaacg tggacagcct catcacccgg
 301 ctgctggagg tacgaggatg tcgtccagga aagattgtgc agatgactga agcagaagtt
 361 cgaggcttat gtatcaagtc tcgggagatc tttctcagcc agcctattct tttggaattg
 421 gaagcaccgc tgaaaatttg tggagatatt catggacaat atacagattt actgagatta
 481 tttgaatatg gaggtttccc accagaagcc aactatcttt tcttaggaga ttatgtggac
 541 agaggaaagc agtctttgga aaccatttgt ttgctattgg cttataaaat caaatatcca
 601 gagaacttct ttctcttaag aggaaaccat gagtgtgcta gcatcaatcg catttatgga
 661 ttctatgatg aatgcaaacg aagatttaat attaaattgt ggaagacctt cactgattgt
 721 tttaactgtc tgcctatagc agccattgtg gatgagaaga tctttctgttg tcatggagga
 781 ttgtcaccag acctgcaatc tatggagcag attcggagaa ttatgagacc tactgatgtc
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 961 ctgaatcgtc atgatttaga tttgatttgt cgagctcatc aggtggtgga agatggatat
1021 gaatttttg ctaaacgaca gttggtaacc ttattttcag ccccaaatta ctgtggcgag
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1141 ttgaaaccat ctgaaaagaa agctaaatac cagtatggtg gactgaattc tggacgtcct
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1261 gaaaccatca gatttgttaa ggacatactt cataatatat aagtgtgcac tgtaaaacca
1321 tccagccatt tgacaccctt tatgatgtca caccttaac ttaaggagac gggtaaagga
1381 tcttaaattt ttttctaata gaaagatgtg ctacactgta ttgtaataag tatactctgt
1441 tatagtcaac aaagttaaat ccaaattcaa aattatccat taaagttaca tcttcatgta
1501 tcacaatttt taaagttgaa aagcatccca gttaaactag atgtgatagt taaaccagat
1561 gaaagcatga tgatccatct gtgtaatgtg gttttagtgt tgcttggttg tttaattatt
1621 ttgagcttgt tttgtttttg tttgttttca ctagaataat ggcaaatact tctaattttt
1681 ttccctaaac atttttaaaa gtgaaatatg ggaagagctt tacagacatt caccaactat
1741 tattttccct tgtttatcta cttagatatc tgtttaatct tactaagaaa actttcgcct
1801 cattacatta aaaaggaatt ttagagattg attgttttaa aaaaaaatac gcacattgtc
1861 caatccagtg attttaatca tacagtttga ctgggcaaac tttacagctg atagtgaata
1921 ttttgcttta tacaggaatt gacactgatt tggatttgtg cactctaatt tttaacttat
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2041 tagttggtaa tgtgattatg tggtaccttg gctttaggtt ttcattcgca cggaacacct
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2161 ctttctttt ttttttttt tttttttga gttgttgttt gtttttagat ccacagtaca
2221 tgagaatcct tttttgacaa gccttggaaa gctgacactg tctcttttc ctccctctat
2281 acgaaggatg tatttaaatg aatgctggtc agtgggacat tttgtcaact atgggtattg
2341 ggtgcttaac tgtctaatat tgccatgtga atgttgtata cgattgtaag gcttatgtca
2401 ctaaagattt ttattctgat tttttcataa tcaaaggtca tatgatactg tatagacaag
2461 ctttgtagtg aagtatagta gcaataattt ctgtacctga tcaagtttat tgcagccttt
2521 cttttcctat ttctttttt taagggttag tattaacaaa tggcaatgag tagaaaagtt
2581 aacatgaaga ttttagaagg agagaactta caggacacag atttgtgatt ctttgactgt
2641 gacactattg gatgtgattc taaaagcttt tattgagcat tgtcaaattt gtaagcttca
2701 tagggatgga catcatatct ataatgccct tctatatgtg ctaccataga tgtgacattt
2761 ttgaccttaa tatcgtcttt gaaaatgtta aattgagaaa cctgttaact tacattttat
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2881 ttctttaaaa tgcatatgtc tttttttcta attccgtttt gttttaaagc acattttaaa
2941 tgtagttttc tcatttagta aaagttgtct aattgatatg aagcctgact gatttttttt
3001 ttccttacag tgagacattt aagcacacat tttattcaca tagatactat gtccttgaca
3061 tattgaaatg attcttttct gaaagtattc atgatctgca tatgatgtat taggttaggt
3121 cacaaaggtt ttatctgagg tgatttaaat aacttcctga ttggagtgtg taagctgagc
3181 gatttctaat aaaatttttag ttgtacactt ttagtagtca tagtgaagca ggtctagaaa
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3301 tctgtatccc attgtctggc ttttgtaaat tcatccaggt caagactaag tatgttggtt
3361 aataggaatc cttttttttt tttaaagact aaatgtgaaa aaataatcac tacttaagct
```

Figure 10 (cont.)

```
3421 aattaatatt ggtcattaaa tttaaaggat ggaaatttat catgtttaaa aattattcaa
3481 gcactcttaa aaccacttaa acagcctcca gtcataaaaa tgtgttcttt acaaatattt
3541 gcttggcaac acgacttgaa ataaataaaa ctttgtttct taggagaaaa

SEQ ID NO:80
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  61 gggctgggcc tgagcatcgt tgggggttca gacacgctgc tgggtgccat tattatccat
 121 gaagtttatg aagaaggagc agcatgtaaa gatggaagac tctgggctgg agatcagatc
 181 ttagaggtga atggaattga cttgagaaag gccacacatg atgaagcaat caatgtcctg
 241 agacagacgc cacagagagt gcgcctgaca ctctacagag atgaggcccc atacaaagag
 301 gaggaagtgt gtgacaccct cactattgag ctgcagaaga agccgggaaa aggcctagga
 361 ttaagtattg ttggtaaaag aaacgatact ggagtatttg tgtcagacat tgtcaaagga
 421 ggaattgcag atgccgatgg aagactgatg cagggagacc agatattaat ggtgaatggg
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 601 tctcaaagca gccaggtgag tgaaggcagc ctgtcatctt tcacttttcc actctctgga
 661 tccagtacat ctgagtcact ggaaagtagc tcaaagaaga atgcattggc atctgaaata
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1141 ggcttcagta tagttggagg atatggcagc cctcatggag acttacccat ttatgttaaa
1201 acagtgtttg caaagggagc agcctctgaa gacggacgtc tgaaaagggg cgatcagatc
1261 attgctgtca atgggcagag tctagaagga gtcacccatg aagaagctgt tgccatcctt
1321 aaacggacaa aaggcactgt cactttgatg gttctctctt gaattggctg ccagaattga
1381 accaacccaa cccctagctc acctcctact gtaaagagaa tgcactggtc ctgacaattt
1441 ttatgctgtg ttcagccggg tcttcaaaac tgtaggggag aaataacact taagtttctt
1501 tttctcatct agaaatgctt tccttactga caacctaaca tcatttttct tttcttcttg
1561 cattttgtga acttaaagag aaggaatatt tgtgtaggtg aatctcgttt ttatttgtgg
1621 agatatctaa tgttttgtag tcacatgggc aagaattatt acatgctaag ctggttagta
1681 taaagaaaga taattctaaa gctaaccaaa gaaaatggct tcagtaaatt aggatgaaaa
1741 atgaaaatat

SEQ ID NO:81
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 121 atacaatggt gcagcagaaa ttggatgata tcaaggatcg aattaagaga gaaataagga
 181 aagaactgaa aatcaaagaa ggagctgaaa atctgaggaa agtcacaaca gataaaaaaa
 241 gtttggctta tgtagacaac attttgaaaa aatcaaataa aaaattagaa gaactacatc
 301 acaagctgca ggaattaaat gcacatattg ttgtatcaga tccagaagat attacagatt
 361 gcccaaggac tccagatact ccaaataatg accctcgttg ttctactagc aacaatagat
 421 tgaaggcctt acaaaaacaa ttggatatag aacttaaagt aaaacaaggt gcagagaata
 481 tgatacagat gtattcaaat ggatcttcaa aggatcggaa actccatggt acagctcagc
 541 aactgctcca ggacagcaag acaaaaatag aagtcatacg aatgcagatt cttcaggcag
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 661 ggatggaaga attaaggcat cattttagga tagagtttgc agtagcagaa ggtgcaaaga
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 781 aagcaagatt taatgaatca agtcagaagt tggacctttt aaagtattca ttagagcaaa
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 901 ttgttgctgc atcaccaaca ctaagtccac gtcaaagtat gatatctacg caaaatcaat
 961 atagtacact atccaaacca gcagcactaa caggtacttt ggaagttcgt cttatgggct
```

Figure 10 (cont.)

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1021 gccaagatat cctagagaat gtccctggac ggtcaaaagc aacatcagtt gcactgcctg
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1141 gaagtagtcg aaatcttcta aaaaccgatg acttgtccaa tgatgtctgt gctgttttga
1201 agctcgataa tactgtggtt ggccaaacta gctggaaacc catttccaat cagtcatggg
1261 accagaagtt tacactggaa ctggacaggt cacgtgaact ggaaatttca gtttattggc
1321 gtgattggcg gtctctgtgt gctgtaaaat ttctgaggtt agaagatttt ttagacaacc
1381 aacggcatgg catgtgtctc tatttggaac cacagggtac tttatttgca gaggttacct
1441 tttttaatcc agttattgaa agaagaccaa aacttcaaag acaaaagaaa atttttttcaa
1501 agcaacaagg caaaacattt ctcagagctc ctcaaatgaa tattaatatt gccacttggg
1561 gaaggctagt aagaagagct attcctacag taaatcattc tggcaccttc agccctcaag
1621 ctcctgtgcc tactacagtg ccagtggttg atgtacgcat ccctcaacta gcacctccag
1681 ctagtgattc tacagtaacc aaattggact ttgatcttga gcctgaacct cctccagccc
1741 caccacgagc ttcttctctt ggagaaatag atgaatcttc tgaattaaga gttttggata
1801 taccaggaca ggattcagag actgtttttg atattcagaa tgacagaaat agtatacttc
1861 caaaatctca atctgaatac aagcctgata ctcctcagtc aggcctagaa tatagtggta
1921 ttcaagaact tgaggacaga agatctcagc aaaggtttca gtttaatcta caagatttca
1981 ggtgttgtgc tgtcttggga agaggacatt ttggaaaggt gcttttagct gaatataaaa
2041 acacaaatga gatgtttgct ataaaagcct taaagaaagg agatattgtg gctcgagatg
2101 aagtagacag cctgatgtgt gaaaaaagaa tttttgaaac tgtgaatagt gtaaggcatc
2161 cctttttggt gaacctttt gcatgtttcc aaaccaaaga gcatgtttgc tttgtaatgg
2221 aatatgctgc cggtggggac ctaatgatgc acattcatac tgatgtcttt tctgaaccaa
2281 gagctgtatt ttatgctgct tgtgtagttc ttgggttgca gtatttacat gaacacaaaa
2341 ttgtttatag agatttgaaa ttggataact tattgctaga tacagagggc tttgtgaaaa
2401 ttgctgattt tggtctttgc aaagaaggaa tgggatatgg agatagaaca agcacatttt
2461 gtggcactcc tgaatttctt gccccagaag tattaacaga aacttcttat acaagggctg
2521 tagattggtg gggccttggc gtgcttatat atgaaatgct tgttggtgag tctccctttc
2581 ctggtgatga tgaagaggaa gtttttgaca gtattgtaaa tgatgaagta aggtatccaa
2641 ggttcttatc tacagaagcc atttctataa tgagaaggct gttaagaaga aatcctgaac
2701 ggcgccttgg ggctagcgag aaagatgcag aggatgtaaa aaagcaccca tttttccggc
2761 taattgattg gagcgctctg atggacaaaa aagtaaagcc accatttata cctaccataa
2821 gaggacgaga agatgttagt aattttgatg atgaatttac ctcagaagca cctattctga
2881 ctccacctcg agaaccaagg atactttcgg aagaggagca ggaaatgttc agagattttg
2941 actacattgc tgattggtgt taagttgcta gacactgcga aaccaagctg actcacaaga
3001 agacctctta aaaatagcaa cccttcattt gctctctgtg ccaccaatag cttctgagtt
3061 ttttgttgtt gttgttttta ttgaaacacg tgaagatttg tttaaaagta ccattctaat
3121 acttcttcaa aagtggctcc tcattgtact tcagcgtaaa tatgagcact ggaaacagtt
3181 tcatggagtt taagttgagt gaacatcggc catgaaaatc catcacgaat acttttggat
3241 caatagtcta tttt
```

SEQ ID NO:82

```
   1 atgaaattca agttacatgt gaattctgcc aggcaataca aggacctgtg gaatatgagt
  61 gatgacaaac cctttctatg tactgcgcct ggatgtggcc agagtgaagt caccctgctg
 121 agaaatgaag tggcacagct gaaacagctt cttctggctc ataaagattg ccctgtaacc
 181 gccatgcaga agaaatctgg ctatcatact gctgataaag atgatagttc agaagacatt
 241 tcagtgccga gtagtccaca tacagaagct atacagcata gttcggtcag cacatccaat
 301 ggagtcagtt caacctccaa ggcagaagct gtagccactt cagtcctcac ccagatggcg
 361 gaccagagta cagagcctgc tctttcacag atcgttatgg ctccttcctc ccagtcacag
 421 ccctcaggaa gttgattaaa aacctgcagt acaacagttt tagatactca ttagtgactt
 481 caaagggaaa tcaaggaaag accagtttc
```

SEQ ID NO:83

```
   1 gaattctgga agttcattga agagtctgaa attagggact tatttcaaat ttggacatgg
  61 ctagtcgagg cgcaacaaga cccaacggcc caaatactgg aaataaaata tgccagttca
 121 aactagtact tctgggagag tccgctgttg gcaaatcaag cctagtgctt cgttttgtga
```

Figure 10 (cont.)

```
       181 aaggccaatt tcatgaattt caagagagta ccattggggc tgcttttcta acccaaactg
       241 tatgtcttga tgacactaca gtaaagtttg aaatctggga tacagctggt caagaaggat
       301 accatagcct agcaccaatg tactacagag gagcacaagc agccatagtt gtatatgata
       361 tcacaaatga ggagtccttt gcaagagcaa aaaattgggt taaagaactt cagaggcaag
       421 caagtcctaa cattgtaata gctttatcgg gaaacaaggc cgacctagca aataaaagag
       481 cagtagattt ccaggaagca cagtcctatg cagatgacaa tagtttatta ttcatggaga
       541 catccgctaa aacatcaatg aatgtaaatg aaatattcat ggcaatagct aaaaaattgc
       601 caaagaatga accacaaaat ccaggagcaa attctgccag aggaggagga gtagacctta
       661 ccgaacccac acaaccaacc aggaatcagt gttgtagtaa ctaaacctct agtttgaac

SEQ Id NO:84
         1 gacgctctgg gccgccacct ccgcggaccc tgagcgcaag agccaagccg ccagcgctgc
        61 gatgtgggcc acgctgccgc tgctctgcgc cggggcctgg ctcctgggag tccccgtctg
       121 cggtgccgcc gaactgtgcg tgaactcctt agagaagttt cacttcaagt catggatgtc
       181 taagcaccgt aagacctaca gtacggagga gtaccaccac aggctgcaga cgtttgccag
       241 caactggagg aagataaacg cccacaacaa tgggaaccac acatttaaaa tggcactgaa
       301 ccaattttca gacatgagct ttgctgaaat aaaacacaag tatctctggt cagagcctca
       361 gaattgctca gccaccaaaa gtaactacct tcgaggtact ggtccctacc caccttccgt
       421 ggactggcgg aaaaaaggaa attttgtctc acctgtgaaa aatcagggtg cctgcggcag
       481 ttgctggact ttctccacca ctggggccct ggagtctgcg atcgccatcg caaccggaaa
       541 gatgctgtcc ttggcggaac agcagctggt ggactgcgcc caggacttca ataatcacgg
       601 ctgccaaggg ggtctcccca gccaggcttt cgagtatatc ctgtacaaca aggggatcat
       661 gggtgaagac acctacccct accagggcaa ggatggttat tgcaagttcc aacctggaaa
       721 ggccatcggc tttgtcaagg atgtagccaa catcacaatc tatgacgagg aagcgatggt
       781 ggaggctgtg gccctctaca accctgtgag ctttgccttt gaggtgactc aggacttcat
       841 gatgtataga accggcatct actccagtac ttcctgccat aaaactccag ataaagtaaa
       901 ccatgcagta ctggctgttg ggtatggaga aaaaaatggg atcccttact ggatcgtgaa
       961 aaactcttgg ggtccccagt ggggaatgaa cgggtacttc ctcatcgagc gcggaaagaa
      1021 catgtgtggc ctggctgcct gcgcctccta ccccatccct ctggtgtgag ccgtggcagc
      1081 cgcagcgcag actggcggag aaggagagga acgggcagcc tgggcctggg tggaaatcct
      1141 gccctggagg aagttgtggg gagatccact gggacccccа acattctgcc ctcacctctg
      1201 tgcccagcct ggaaacctac agacaaggag gagttccacc atgagctcac ccgtgtctat
      1261 gacgcaaaga tcaccagcca tgtgccttag tgtccttctt aacagactca aaccacatgg
      1321 accacgaata ttctttctgt ccagaagggc tactttccac atatagagct ccagggactg
      1381 tcttttctgt attcgctgtt caataaacat tgagtgagca cctccccaga tgg

SEQ ID NO:85
         1 ggtcggggcc cgcggccgct cgcgcctctc gatgggcagc tcgcacttgc tcaacaaggg
        61 cctgccgctt ggcgtccgac ctccgatcat gaacgggccc ctgcacccgc ggcccctggt
       121 ggcattgctg gatggccggg actgcacagt ggagatgccc atcctgaagg acgtggccac
       181 tgtggccttc tgcgacgcgc agtccacgca ggagatccat gagaaggtcc tgaacgaggc
       241 tgtgggggcc ctgatgtacc acaccatcac tctcaccagg gaggacctgg agaagttcaa
       301 agccctccgc atcatcgtcc ggattggcag tggttttgac aacatcgaca tcaagtcggc
       361 cggggattta ggcattgccg tctgcaacgt gcccgcggcg tctgtggagg agacggccga
       421 ctcgacgctg tgccacatcc tgaacctgta ccggcgggcc acctggctgc accaggcgct
       481 gcgggagggc acacgagtcc agagcgtcga gcagatccgc gaggtggcgt ccggcgctgc
       541 caggatccgc ggggagacct tgggcatcat cggacttgtc gcgtggggca ggcagtggcg
       601 ctgcgggcca aggccttcgg cttcaacgtg ctcttctacg acccttactt gtcggatggc
       661 gtggagcggg cgctggggct gcagcgtgtc agcaccctgc aggacctgct cttccacagc
       721 gactgcgtga ccctgcactg cggcctcaac gagcacaacc accacctcat caacgacttc
       781 accgtcaagc agatgagaca aggggccttc ctggtgaaca cagcccgggg tggcctggtg
       841 gatgagaagg cgctggccca ggccctgaag gagggccgga tccgcggcgc ggccctggat
       901 gtgcacgagt cggaaccctt cagctttagc cagggccctc tgaaggatgc acccaacctc
       961 atctgcaccc cccatgctgc atggtacagc gagcaggcat ccatcgagat gcgagaggag
```

Figure 10 (cont.)

```
1021 gcggcacggg agatccgcag agccatcaca ggccggatcc cagacagcct gaagaactgt
1081 gtcaacaagg accatctgac agccgccacc cactgggcca gcatggaccc cgccgtcgtg
1141 caccctgagc tcaatggggc tgcctatagg taccctccgg gcgtggtggg cgtggccccc
1201 actggcatcc cagctgctgt ggaaggtatc gtccccagcg ccatgtccct gtcccacggc
1261 ctgccccctg tggcccaccc gccccacgcc ccttctcctg gccaaaccgt caagcccgag
1321 gcggatagag accacgccag tgaccagttg tagcccggga ggagctctcc agcctcggcg
1381 cctgggcaga gggcccggaa accctcggac cagagtgtgt ggaggaggca tctgtgtggt
1441 ggccctggca ctgcagagac tggtccgggc tgtcaggagg cgggaggggg cagcgctggg
1501 cctcgtgtcg cttgtcgtcg tccgtcctgt gggcgctctg ccctgtgtcc ttcgcgttcc
1561 tcgttaagca gaagaagtca gtagttattc tcccatgaac gttcttgtct gtgtacagtt
1621 tttagaacat tacaaaggat ctgtttgctt agctgtcaac aaaaagaaaa cctgaaggag
1681 catttggaag tcaatttgag gttttttttt ttgttttttt ttttttgta tgttggaacg
1741 tgccccagaa tgaggcagtt ggcaaacttc tcaggacaat gaatccttcc cgtttttctt
1801 tttatgccac acagtgcatt gttttttcta cctgcttgtc ttatttttag aataatttag
1861 aaaaacaaaa caaaggctgt ttttcctaat tttggcatga acccccccct gttccaaatg
1921 aagacggcat cacgaagcag ctccaaaagg aaaagcttgg gcggtgccca gcgtgcccgc
1981 tgcccatcga cgtctgtcct ggggacgtgg agggtggcag cgtccccgcc tgcaccagtg
2041 ccgtcctgct gatgtggtag gctagcaata ttttggttaa aatcatgttt gtg
```

SEQ ID NO:86

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   1 cgcgcggcca ggccctctta gccctctgcc gtttgggggg cacgggtgaa cctgccgccc
  61 cactcccacc ccgccccgcc ccgcccgtac agccaaatcg gaagggacga gcctgccctt
 121 tgaaagggtt ttttttcttg ctcctgcgga gggcgcccca gccatggccc tcaggagctc
 181 cctagacccc gcagggactg ccctccatcc cggccgccgg ggcccgccct ctgcatcccg
 241 cgggcagcct gtgtgaagcg gcctcccgca gcccccggcc cctcccccat ggaggaggag
 301 gagggggcgg tggccaagga gtggggcacg acccccgcgg ggcccgtctg gaccgcggtg
 361 ttcgactacg aggcggcggg cgacgaggag ctgaccctgc ggaggggcga tcgcgtccag
 421 gtgctttccc aagactgtgc ggtgtccggc gacgagggct ggtggaccgg gcagctcccc
 481 agcggccgcg tgggcgtctt ccccagcaac tacgtggccc ccggcgcccc cgctgcaccc
 541 gcgggcctcc agctgcccca ggagatcccc ttccacgagc tgcagctaga ggagatcatc
 601 ggtgtggggg gctttggcaa ggtctatcgg gccctgtggc gtggcgagga ggtggcagtc
 661 aaggccgccc ggctggaccc tgagaaggac ccggcagtga cagcggagca ggtgtgccag
 721 gaagcccggc tctttggagc cctgcagcac cccaacataa ttgcccttag gggcgcctgc
 781 ctcaaccccc cacacctctg cctagtgatg gagtatgccc ggggtggtgc actgagcagg
 841 gtgctggcag gtcgccgggt gccacctcac gtgctggtca actgggctgt gcaggtggcc
 901 cggggcatga actacctaca caatgatgcc cctgtgccca tcatccaccg ggacctcaag
 961 tccatcaaca tcctgatcct ggaggccatc gagaaccaca acctcgcaga cacggtgctc
1021 aagatcacgg acttcggcct cgcccgcgag tggcacaaga ccaccaagat gagcgctgcg
1081 gggacctacg cctggatggc gccggaggtt atccgtctct ccctcttctc caaaagcagt
1141 gatgtctgga gcttcggggt gctgctgtgg gagctgctga cggggaggt cccctaccgt
1201 gagatcgacg ccttggccgt ggcgtatggc gtggctatga ataagctgac gctgcccatt
1261 ccctccacgt gccccgagcc ctttgcccgc ctcctggagg aatgctggga cccagacccc
1321 cacgggcggc cagatttcgg tagcatcttg aagcggcttg aagtcatcga acagtcagcc
1381 ctgttccaga tgccactgga gtccttccac tcgctgcagg aagactggaa gctggagatt
1441 cagcacatgt ttgatgacct tcggaccaag gagaaggagc ttcggagccg tgaggaggag
1501 ctgctgcggg cggcacagga gcagcgcttc caggaggagc agctgcggcg gcgggagcag
1561 gagctggcag aacgtgagat ggacatcgtg gaacgggagc tgcacctgct catgtgccag
1621 ctgagccagg agaagccccg ggtccgcaag cgcaagggca acttcaagcg cagccgcctg
1681 ctcaagctgc gggaaggcgg cagccacatc agcctgccct ctggctttga gcataagatc
1741 acagtccagg cctctccaac tctggataag cggaaaggat ccgatggggc cagccccct
1801 gcaagcccca gcatcatccc ccggctgagg gccattcgcc tgactcccgt ggactgtggt
1861 ggcagcagca gtggcagcag cagtggagga agtgggacat ggagccgcgg tgggccccca
1921 aagaaggaag aactggtcgg gggcaagaag aagggacgaa cgtgggggcc cagctccacc
1981 ctgcagaagg agcgggtggg aggagaggag aggctgaagg ggctgggga aggaagcaaa
```

Figure 10 (cont.)

```
2041 cagtggtcat caagtgcccc caacctgggc aagtccccca aacacacacc cagtcgccgc
2101 tggcttcgcc agcctcaatg agatggagga gttcgcggag gcagaggatg gaggcagcag
2161 cgtgccccct tccccctact cgaccccgtc ctacctctca gtgccactgc ctgccgagcc
2221 ctccccgggg gcgcgggcgc cgtgggagcc gacgccgtcc gcgccccccg ctcggtgggg
2281 acacggcgcc cggcggcgct gcgacctggc gctgctaggc tgcgccacgc tgctgggggc
2341 tgtgggcctg ggcgccgacg tggccgaggc gcgcgcggcc gacggtgagg agcagcggcg
2401 ctggctcgac ggcctcttct ttccccgcgc cggccgcttc ccgcggggcc tcagcccacc
2461 cgcgcgtccc cacggccgcc gcgaagacgt gggcccccggc ctgggcctgg cgccctcggc
2521 caccctcgtg tcgctgtcgt ccgtgtccga ctgcaactcc acgcgttcac tgctgcgctc
2581 tgacagtgac gaggccgcac cggccgcgcc ctccccacca ccctccccgc ccgcgcccac
2641 acccacgccc tcgcccagca ccaacccccct ggtggacctg gagctggaga gcttcaagaa
2701 ggaccccccgc cagtcgctca cgcccaccca cgtcacggct gcatgcgctg tgagccgcgg
2761 gcaccggcgg acgccatcgg atggggcgct ggggcagcgg gggccgcccg agcccgcggg
2821 ccatggccct ggccctcgtg accttctgga cttcccccgc ctgcccgacc cccaggccct
2881 gttcccagcc cgccgccggc cccctgagtt cccaggccgc cccaccaccc tgacctttgc
2941 cccgagacct cggccggctg ccagtcgccc ccgcttggac ccctggaaac tggtctcctt
3001 cggccggaca ctcaccatct cgcctcccag caggccagac actccggaga gccctgggcc
3061 ccccagcgtg cagcccacac tgctggacat ggacatggag gggcagaacc aagacagcac
3121 agtgcccctg tgcggggccc acggctccca ctaaggcctg cccaccaccg cccgcctggg
3181 cagccatgaa tgtagcgccc caggccctgc cccagcccgc catgccacaa ggtgggggag
3241 gccctgggca ggatgttcac tctatttatt ggggaaggag ggaggggggg gacacttaac
3301 ttattccttt gtaccccagg gggtggagcc ctgtgcccac cctgcactgg ggggagggtg
3361 ggcagggata ctcagggaca gggcatcatg ggggatttgg cacaaaatgg agcattaaag
3421 gtaaccccctg ccccc
```

SEQ ID NO:87

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   1 gggcccgccc ctggtcacag ccagactgac tcagtttccc tgggaggtcc cgctcgagcc
  61 cgtccttccc ctccctctgc ccgcccccag ccctcgcccc accctcggcg cccgcacatc
 121 tgcctgctca gctccagacg gcgcccggac ccccgggcgc gggatccagc caggtgggag
 181 ccccgcagat gaggtctctg aaggtgtgcc tgaaccagtg ccagcctgcc ctgtctgcag
 241 catcggcctg atggggtggt gactgatccc tcagggctcc ggagccatgt ggcccaacgg
 301 cagttccctg gggccctgtt tccggcccac aaacattacc ctggaggaga gacggctgat
 361 cgcctcgccc tggttcgccg cctccttctg cgtggtgggc ctggcctcca acctgctggc
 421 cctgagcgtg ctggcgggcg cgcggcaggg gggttcgcac acgcgctcct ccttcctcac
 481 cttcctctgc ggcctcgtcc tcaccgactt cctggggctg ctggtgaccg gtaccatcgt
 541 ggtgtcccag cacgccgcgc tcttcgagtg gcacgccgtg gaccctggct gccgtctctg
 601 tcgcttcatg ggcgtcgtca tgatcttctt cggcctgtcc ccgctgctgc tgggggccgc
 661 catggcctca gagcgctacc tgggtatcac ccggcccttc tcgcgcccgg cggtcgcctc
 721 gcagcgccgc gcctgggcca ccgtggggct ggtgtgggcg gccgcgctgg cgctgggcct
 781 gctgcccctg ctgggcgtgg gtcgctacac cgtgcaatac ccggggtcct ggtgcttcct
 841 gacgctgggc gccgagtccg gggacgtggc cttcgggctg ctcttctcca tgctgggcgg
 901 cctctcggtc gggctgtcct tcctgctgaa cacggtcagc gtggccaccc tgtgccacgt
 961 ctaccacggg caggaggcgg cccagcagcg tccccgggac tccgaggtgg agatgatggc
1021 tcagctcctg gggatcatgg tggtggccag cgtgtgttgg ctgccccttc tggtcttcat
1081 cgcccagaca gtgctgcgaa acccgcctgc catgagcccc gccgggcagc tgtcccgcac
1141 cacggagaag gagctgctca tctacttgcg cgtggccacc tggaaccaga tcctggaccc
1201 ctgggtgtat atcctgttcc gccgcgccgt gctccggcgt ctccagcctc gcctcagcac
1261 ccggcccagg tcgctgtccc tccagcccca gctcacgcag cgctccgggc tgcagtagga
1321 agtggacaga gcgcccctcc cgcgcctttc cgcggagccc ttggcccctc ggacagccca
1381 tctgcctgtt ctgaggattc aggggctggg ggtgctggat ggacagtggg catcagcagc
1441 agggttttgg gttgacccca atccaacccg gggacccccca actcctccct gatcctttta
1501 ccaagcactc tcccttcctc ggccccttt tcccatccag agctcccacc ccttctctgc
1561 gtccctccca accccaggaa gggcatgcag acattggaag agggtcttgc attgctattt
1621 ttttttttag acggagtctt gctctgtccc ccaggctgga gtgcagtggc gcaatctcag
```

Figure 10 (cont.)

```
1681 ctcactgcaa cctccacctc ccgggttcaa gcgattctcc tgcctcagcc tcctgagtag
1741 ctgggactat aggcgcgcgc caccacgccc ggctaatttt tgtatttta gtagagacgg
1801 ggtttcaccg tgttggccag gctggtcttg aactcctgac ctcaggtgat tcaccagcct
1861 cagcctccca aagtgctggg atcacaggca tgaaccacca cacctggcca ttttttttt
1921 ttttttaga cggagtctca ctctgtggcc cagcctggag tacagtggca cgatctcggc
1981 tcactgcaac ctccgcctcc cgggttcaag cgattctcgt gcctcagcct cccgagcagc
2041 tgggattaca ggcgtaagcc actgcgcccg gccttgcatg ctctttgacc ctgaatttga
2101 cctacttgct ggggtacagt tgcttccttt tgaacctcca acagggaagg ctctgtccag
2161 aaaggattga atgtgaacgg gggcaccccc ttttcttgcc aaaatatatc tctgcctttg
2221 gttttat
```

SEQ ID NO:88

```
   1 cccggacatg gccgccaaca tgtacagggt cggagactac gtctactttg agaactcctc
  61 cagcaaccca tacctgatcc ggagaatcga ggagctcaac aagacggcca atgggaacgt
 121 ggaggccaaa gtggtgtgct tctaccggag gcgggacatc tccagcaccc tcatcgccct
 181 ggccgacaag cacgcaaccc tgtcagtctg ctataaggcc ggaccggggg cggacaacgg
 241 cgaggaaggg gaaatagaag aggaaatgga gaatccggaa atggtggacc tgcccgagaa
 301 actaaagcac cagctgcggc atcgggagct gttcctctcc cggcagctgg agtctctgcc
 361 cgccacgcac atcaggggca agtgcagcgt caccctgctc aacgagaccg agtcgctcaa
 421 gtcctacctg gagcgggagg atttcttctt ctattctcta gtctacgacc cacagcagaa
 481 gaccctgctg gcagataaag gagagattcg agtaggaaac cggtaccagg cagacatcac
 541 cgacttgtta aaagaaggcg aggaggatgg ccgagaccag tccaggttgg agacccaggt
 601 gtgggaggcg cacaacccac tcacagacaa gcagatcgac cagttcctgg tggtggcccg
 661 ctctgtgggc accttcgcac gggccctgga ctgcagcagc tccgtccgac agcccagcct
 721 gcacatgagc gccgcagctg cctcccgaga catcaccctg ttccacgcca tggatactct
 781 ccacaagaac atctacgaca tctccaaggc catctcggcg ctggtgccgc agggcgggcc
 841 cgtgctctgc agggacgaga tggaggagtg gtctgcatca gaggccaacc ttttcgagga
 901 agccctggaa aaatatggga aggatttcac ggacattcag caagattttc tcccgtggaa
 961 gtcgctgacc agcatcattg agtactacta catgtggaag accaccgaca gatacgtgca
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1081 ctataacaag ccaaatccga accaaatcag cgtcaacaac gtcaaggccg gtgtggtgaa
1141 cggcacgggg gcgccgggcc agagccctgg ggctggccgg gcctgcgaga gctgttacac
1201 cacacagtct taccagtggt attcttgggg tccccctaac atgcagtgtc gtctctgcgc
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1321 agagaggcca ggaccaaacc gcagtaacat gagtccccac ggcctcccag cccggagcag
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1561 agcctcccag agcccgctgg tgctgaagca ggcggtacgc aagccgctgg aagccgtgct
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1681 cgtgctcagc agcctgacgc ccgccaaggt ggcccccgtc atcaacaacg gctcccccac
1741 catcctgggc aagcgcagct acgagcagca caacggggtg gacggcaaca tgaagaagcg
1801 cctcttgatg cccagtaggg gtctggcaaa ccacggacag accaggcaca tgggaccaag
1861 ccggaacctc ctgctcaacg ggaagtccta ccccaccaaa gtgcgcctga tccgggggcg
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2341 gccgaggagt tgtcgttttt agctttgtgt ttacttttg gctggagcgg agatgagggg
2401 ccaccccgtg cccctgtgct gcggggcctt ttgcccggag gccgggccct aaggttttgt
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```

Figure 10 (cont.)

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2521 ttgagctctg taacttacac ctggaatgtt aggatcgtgc ggccgcggcc ggccgagctg
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2641 aaaaatctta taaaaaggaa aa

SEQ ID NO:89
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 121 cgcaaggaga ttgaggtgga ctcgtcgccg tcggtgctgg agatcctgga tacggcgggc
 181 accgagcagt tcgcgtccat gcgggacctg tacatcaaga acggccaggg cttcatcctg
 241 gtctacagcc tcgtcaacca gcagagcttc caggacatca agcccatgcg ggaccagatc
 301 atccgcgtga agcggtacga gcgcgtgccc atgatcctgg tgggcaacaa ggtggacctg
 361 gagggtgagc gcgaggtctc gtacggggag ggcaaggccc tggctgagga gtggagctgc
 421 cccttcatgg agacgtcggc caaaaacaaa gcctcggtag acgagctatt tgccgagatc
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SEQ ID NO:90
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 121 ggccccaggc gaaacccagc ctggagcttg caggcaggac gactgttcag cacgcagacc
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2041 gacgacccta gggccatggt gggccgcttg aatgagctgc ttgtcaaggc cctggagcga
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Figure 10 (cont.)

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 121 ctccatgatg aaaccctaac gtatctcaat caaggacagt cttatgaaat tcgaatgcta
 181 gacaatagga aacttggaga acttccagaa attaatggca aattggtgaa gagtatattc
 241 cgtgtggtgt tccatgacag aaggcttcag tacactgagc atcagcagct agagggctgg
 301 aggtggaacc gacctggaga cagaattctt gacatagata tcccgatgtc tgtgggtata
 361 atcgatccta gggctaatcc aactcaacta aatacagtgg agttcctgtg ggaccctgca
 421 aagaggacat ctgtgtttat tcaggtgcac tgtattagca cagagttcac tatgaggaaa
 481 catggtggag aaaaggggat gccattccga gtacaaatag ataccttcaa ggagaatgaa
 541 aacggggaat atactgagca cttacactcg gccagctgcc agatcaaagt tttcaagcca
 601 aaggtgcaga cagaaagcaa aaaacggata gggaaaaaat ggagaaacga acacctcatg
 661 aaaaggagaa atatcagcct tcctatgaga caaccatact cacagagtgt tctccatggc
 721 ccgagatcac gtatgtcaat aactccccat cacctggctt caacagttcc catagcagtt
 781 tttctcttgg ggaaggaaat ggttcaccaa accaccagcc agagccaccc cctccagtca
 841 cagataacct cttaccaaca accacacctc aggaagctca gcagtggttg catcgaaatc
 901 gttttttctac attcacaagg cttttcacaa acttctcagg ggcagattta ttgaaattaa
 961 ctagagatga tgtgatccaa atctgtggcc ctgcagatgg aatcagactt tttaatgcat
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1081 tgagggagca gcaacaacag cagcagcaac agcagcagaa gcatgaggat ggagactcaa
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1381 tgaagtagga gtgcggcgtt tcgtgcccag tggctgctcc ttccttcacc tctgaaaacg
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1561 gctcttgtgg tacacacaga ttactgccca atatgcagtt ctgcagctgt tttagttaaa
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1681 agggcataat gatgatggga attgtgtgat gtttaacagg aagatcttaa attttgtgat
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 181 gtgcctgctg atccacccta accccgagtc tgcactcaac gaggaggcgg gccgcctgct
 241 cttggagaac tacgaggagt atgcggctcg ggcccgtctg ctcacagaga tccacggggg
 301 cgccggcggg cccagcggca gggccaaagc cgggcgggcc ctggccagtg gcactgcagc
 361 ttcctccacc gactctgggg ccccaggggg cttgggaggg gctgagggtc ccatggccaa
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 541 gtcccctccc tccaactctg tctctaagtt atttaaatta tggctggggt cggggagggt
 601 acaggggggca ctgagacctg gatttgtttt tttaaataaa gttggaaaag ca


SEQ ID NO:93
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 181 cgataaaggg atttctgcct tccctgaatc agacaacctt ttcaaatggg tagggaccat
 241 ccatggagca gctggaacag tatatgaaga cctgaggtat aagctctcgc tagagttccc
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Figure 10 (cont.)

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 301 cagtggctac ccttacaatg cgcccacagt gaagttcctc acgccctgct atcaccccaa
 361 cgtggacacc cagggtaaca tatgcctgga catcctgaag gaaaagtggt ctgccctgta
 421 tgatgtcagg accattctgc tctccatcca gagccttcta ggagaaccca acattgatag
 481 tcccttgaac acacatgctg ccgagctctg gaaaaacccc acagctttta agaagtacct
 541 gcaagaaacc tactcaaagc aggtcaccag ccaggagccc tgacccaggc tgcccagcct
 601 gtccttgtgt cgtcttttta atttttcctt agatggtctg tccttttgt gatttctgta
 661 taggactctt tatcttgagc tgtggtattt ttgttttgtt tttgtctttt aaattaagcc
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SEQ ID NO:94
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 181 tacatcgtgc gccgtggacc aggagaacca agatccaagg agatgggtgc agaaaccacc
 241 gctcaatatt caacgccccc tcgttgattc agcaggcccc aggccgaaag ccaggcacca
 301 ggcagagaca tcacaaagat tggtggggat cagtcagcct cggaacccct tggaagagct
 361 caggcctagc cctaggggtc aaaatgtggg gcctgggccc cctgcccaga cagaggctcc
 421 agggaccata gagtttgtgg ctgaccctgc agccctggcc accatcctgt caggtgaggg
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2341 tacattactc gaatggcagg atgccctgtg tttcattcca gttggttctg ctgccccccа
2401 gggctctcca tgatgagaca accactcctg ccctgccgta cttcttcctt ttagcccttа
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2521 taaagta
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Figure 10 (cont.)

SEQ ID NO:95

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 121 gtaaaggtat gtttagttcc aatcgtcaga aaattttgga aagaacggaa atcttaaacc
 181 aagaatggaa acagcgaagg atacagcctg tgcacatcct gacttctgtg agctcattgc
 241 gcgggactag ggagtgttcg gtgaccagtg acttggattt tccaacacaa gtcatcccat
 301 taaagactct gaatgcagtt gcttcagtac ccataatgta ttcttggtct cccctacagc
 361 agaattttat ggtggaagat gaaactgttt tacataacat tccttatatg ggagatgaag
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 481 acggggatag agaatgtggg tttataaatg atgaaatttt tgtggagttg gtgaatgccc
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 601 aaaagcagaa agatctggag gatcaccgag atgataaaga aagccgccca cctcggaaat
 661 ttccttctga taaaattttt gaagccattt cctcaatgtt tccagataag ggcacagcag
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2401 caacttttta ttgccttctc accagctgca aagtgttttg taccagtgaa tttttgcaat
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SEQ ID NO: 96

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 181 agggtgcgaa caggggcttc gggccacgct tctcttggcg acaggatttt gctgtgaagt
 241 ccgtccggga aacggaggaa aaaaagagtt gcgggaggct gtctgctaat aacggttctt
 301 gatacatatt tgccagactt caagatttca gaaaaggggt gaaagagaag attgcaactt
 361 tgagtcagac ctgtaggcct gatagactga ttaaaccaca gaaggtgacc tgctgagaaa
 421 agtggtacaa atactgggaa aaacctgctc ttctgcgtta agtgggagac aatgtcacaa
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Figure 10 (cont.)

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 601 aagttactgg ggaagaatgg aactggaggg ctttttcagg gcaaaactcc tttgagaaag
 661 gctaatcttc agcaagctat tgtcacacct ttgaaaccag ttgacaacac ttactacaaa
 721 gaggcagaaa aagaaaatct tgtggaacaa tccattccgt caaatgcttg ttcttccctg
 781 gaagttgagg cagccatatc aagaaaaact ccagcccagc ctcagagaag atctcttagg
 841 ctttctgctc agaaggattt ggaacagaaa gaaaagcatc atgtaaaaat gaaagccaag
 901 agatgtgcca ctcctgtaat catcgatgaa attctaccct ctaagaaaat gaaagtttct
 961 aacaacaaaa agaagccaga ggaagaaggc agtgctcatc aagatactgc tgaaaacaat
1021 gcatcttccc cagagaaagc caagggtaga catactgtgc cttgtatgcc acctgcaaag
1081 cagaagtttc taaaaagtac tgaggagcaa gagctggaga agagtatgaa aatgcagcaa
1141 gaggtggtgg agatgcggaa aaagaatgaa gaattcaaga aacttgctct ggctggaata
1201 gggcaacctg tgaagaaatc agtgagccag gtcaccaaat cagttgactt ccacttccgc
1261 acagatgagc gaatcaaaca acatcctaag aaccaggagg aatataagga agtgaacttt
1321 acatctgaac tacgaaagca tccttcatct cctgcccgag tgactaaggg atgtaccatt
1381 gttaagcctt tcaacctgtc ccaaggaaag aaaagaacat ttgatgaaac agtttctaca
1441 tatgtgcccc ttgcacagca agttgaagac ttccataaac gaacccctaa cagatatcat
1501 ttgaggagca agaaggatga tattaacctg ttaccctcca aatcttctgt gaccaagatt
1561 tgcagagacc cacagactcc tgtactgcaa accaaacacc gtgcacgggc tgtgacctgc
1621 aaaagtacag cagagctgga ggctgaggag ctcgagaaat tgcaacaata caaattcaaa
1681 gcacgtgaac ttgatcccag aatacttgaa ggtgggccca tcttgcccaa gaaaccacct
1741 gtgaaaccac ccaccgagcc tattggcttt gatttggaaa ttgagaaaag aatccaggag
1801 cgagaatcaa agaagaaaac agaggatgaa cactttgaat ttcattccag accttgccct
1861 actaagattt tggaagatgt tgtgggtgtt cctgaaaaga aggtacttcc aatcaccgtc
1921 cccaagtcac cagcctttgc attgaagaac agaattcgaa tgcccaccaa agaagatgag
1981 gaagaggacg aaccggtagt gataaaagct caacctgtgc cacattatgg ggtgcctttt
2041 aagccccaaa tcccagaggc aagaactgtg gaaatatgcc ctttctcgtt tgattctcga
2101 gacaaagaac gtcagttaca gaaggagaag aaaataaaag aactgcagaa aggggaggtg
2161 cccaagttca aggcacttcc cttgcctcat tttgacacca ttaacctgcc agagaagaag
2221 gtaaagaatg tgacccagat tgaacctttc tgcttggaga ctgacagaag aggtgctctg
2281 aaggcacaga cttggaagca ccagctggaa gaagaactga gacagcagaa agaagcagct
2341 tgtttcaagg ctcgtccaaa caccgtcatc tctcaggagc cctttgttcc caagaaagag
2401 aagaaatcag ttgctgaggg cctttctggt tctctagttc aggaaccttt tcagctggct
2461 actgagaaga gagccaaaga gcggcaggag ctggagaaga gaatggctga ggtagaagcc
2521 cagaaagccc agcagttgga ggaggccaga ctacaggagg aagagcagaa aaaagaggag
2581 ctggccaggc tacggagaga actggtgcat aaggcaaatc caatacgcaa gtaccagggt
2641 ctggagataa agtcaagtga ccagcctctg actgtgcctg tatctcccaa attctccact
2701 cgattccact gctaaactca gctgtgagct gcggataccg cccggcaatg ggacctgctc
2761 ttaacctcaa acctaggacc gtcttgcttt gtcattgggc atggagagaa cccatttctc
2821 cagactttta cctacccgtg cctgagaaag catacttgac aactgtggac tccagttttg
2881 ttgagaattg ttttcttaca ttactaaggc taataatgag atgtaactca tgaatgtctc
2941 gattagactc catgtagtta cttcctttaa accatcagcc ggccttttat atgggtcttc
3001 actctgacta gaatttagtc tctgtgtcag cacagtgtaa tctctattgc tattgccccc
3061 tacgactctc accctctccc cacttttttt aaaaatttta accagaaaat aaagatagtt
3121 aaatcctaag atagagatta agtcatggtt taaatgagga acaatcagta aatcagattc
3181 tgtcctcttc tctgcatacc gtgaatttat agttaaggat ccctttgctg tgagggtaga
3241 aaacctcacc aactgcacca gtgaggaaga agactgcgtg gattcatggg gagcctcaca
3301 gcagccacgc agcaggctct gggtggggct gccgttaagg cacagttctt tccttactgg
3361 tgctgataac aacagggaac cgtgcagtgt gcattttaag acc
```

SEQ ID NO:97

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   1 cttcaacccg cgccggcggc gactgcagtt cctgcgagcg aggagcgcgg gacctgctga
  61 cacgctgacg ccttcgagcg cggcccgggg cccggagcgg ccggagcagc ccgggtcctg
 121 accccggccc ggctcccgct ccgggctctg ccggcgggcg ggcgagcgcg gcgcggtccg
 181 ggccgggggg atgtctcggc ggacgcgctg cgaggatctg gatgagctgc actaccagga
```

Figure 10 (cont.)

```
 241 cacagattca gatgtgccgg agcagaggga tagcaagtgc aaggtcaaat ggacccatga
 301 ggaggacgag cagctgaggg ccctggtgag gcagtttgga cagcaggact ggaagttcct
 361 ggccagccac ttccctaacc gcactgacca gcaatgccag tacaggtggc tgagagtttt
 421 gaatccagac cttgtcaagg ggccatggac caaagaggaa gaccaaaaag tcatcgagct
 481 ggttaagaag tatggcacaa agcagtggac actgattgcc aagcacctga agggccggct
 541 ggggaagcag tgccgtgaac gctggcacaa ccacctcaac cctgaggtga agaagtcttg
 601 ctggaccgag gaggaggacc gcatcatctg cgaggcccac aaggtgctgg gcaaccgctg
 661 ggccgagatc gccaagatgt tgccagggag gacagacaat gctgtgaaga atcactggaa
 721 ctctaccatc aaaaggaagg tggacacagg aggcttcttg agcgagtcca aagactgcaa
 781 gcccccagtg tacttgctgc tggagctcga ggacaaggac ggcctccaga gtgcccagcc
 841 cacggaaggc cagggaagtc ttctgaccaa ctggccctcc gtccctccta ccataaagga
 901 ggaggaaaac agtgaggagg aacttgcagc agccaccaca tcgaaggaac aggagcccat
 961 cggtacagat ctggacgcag tgcgaacacc agagcccttg gaggaattcc cgaagcgtga
1021 ggaccaggaa ggctccccac cagaaacgag cctgccttac aagtgggtgg tggaggcagc
1081 taacctcctc atccctgctg tgggttctag cctctctgaa gccctggact tgatcgagtc
1141 ggaccctgat gcttggtgtg acctgagtaa atttgacctc cctgaggaac catctgcaga
1201 ggacagtatc aacaacagcc tagtgcagct gcaagcgtca catcagcagc aagtcctgcc
1261 accccgccag ccttccgccc tggtgcccag tgtgaccgag taccgcctgg atggccacac
1321 catctcagac ctgagccgga gcagccgggg cgagctgatc cccatctccc ccagcactga
1381 agtcgggggc tctggcattg gcacaccgcc ctctgtgctc aagcggcaga ggaagaggcg
1441 tgtggctctg tcccctgtca ctgagaatag caccagtctg tccttcctgg attcctgtaa
1501 cagcctcacg cccaagagca cacctgttaa gaccctgccc ttctcgccct cccagtttct
1561 gaacttctgg aacaaacagg acacattgga gctggagagc ccctcgctga catccacccc
1621 agtgtgcagc cagaaggtgg tggtcaccac accactgcac cgggacaaga caccctgca
1681 ccagaaacat gctgcgtttg taaccccaga tcagaagtac tccatggaca acactcccca
1741 cacgccaacc ccgttcaaga acgccctgga gaagtacgga cccctgaagc ccctgccaca
1801 gaccccgcac ctggaggagg acttgaagga ggtgctgcgt tctgaggctg gcatcgaact
1861 catcatcgag gacgacatca ggcccgagaa gcagaagagg aagcctgggc tgcggcggag
1921 ccccatcaag aaagtccgga agtctctggc tcttgacatt gtggatgagg atgtgaagct
1981 gatgatgtcc acactgccca agtctctatc cttgccgaca actgcccctt caaactcttc
2041 cagcctcacc ctgtcaggta tcaaagaaga caacagcttg ctcaaccagg gcttcttgca
2101 ggccaagccc gagaaggcag cagtggccca gaagccccga agccacttca cgacacctgc
2161 ccctatgtcc agtgcctgga agacggtggc ctgcgggggg accagggacc agctttttcat
2221 gcaggagaaa gcccggcagc tcctgggccg cctgaagccc agccacacat ctcggaccct
2281 catcttgtcc tgaggtgttg agggtgtcac gagcccattc tcatgtttac aggggttgtg
2341 ggggcagagg gggtctgtga atctgagagt cattcaggtg acctcctgca gggagccttc
2401 tgccaccagc ccctccccag actctcaggt ggaggcaaca gggccatgtg ctgccctgtt
2461 gccgagccca gctgtgggcg gctcctggtg ctaacaacaa agttccactt ccaggtctgc
2521 ctggttccct ccccaaggcc acagggagct ccgtcagctt ctcccaagcc cacgtcaggc
2581 ctggcctcat ctcagaccct gcttaggatg ggggatgtgg ccaggggtgc tcctgtgctc
2641 accctctctt ggtgcatttt tttggaagaa taaaattgcc tctctctt
```

SEQ ID NO:98

```
   1 atgaggttga cgctactttg ttgcacctgg agggaagaac gtatgggaga ggaaggaagc
  61 gagttgcccg tgtgtgcaag ctgcggccag aggatctatg atggccagta cctccaggcc
 121 ctgaacgcgg actggcacgc agactgcttc aggtgttgtg actgcagtgc ctccctgtcg
 181 caccagtact atgagaagga tgggcagctc ttctgcaaga aggactactg ggcccgctat
 241 ggcgagtcct gccatgggtg ctctgagcaa atcaccaagg gactggttat ggtggctggg
 301 gagctgaagt accacccccga gtgtttcatc tgcctcacgt gtgggacctt tatcggtgac
 361 ggggacacct acacgctggt ggagcactcc aagctgtact gcgggcactg ctactaccag
 421 actgtggtga cccccgtcat cgagcagatc ctgcctgact cccctggctc ccacctgccc
 481 cacaccgtca ccctggtgtc catcccagcc tcatctcatg gcaagcgtgg actttcagtc
 541 tccattgacc ccccgcacgg cccaccgggc tgtggcaccg agcactcaca caccgtccgc
 601 gtccagggag tggatccggg ctgcatgagc ccagatgtga agaattccat ccacgtcgga
```

Figure 10 (cont.)

```
 661 gaccggatct tggaaatcaa tggcacgccc atccgaaatg tgcccctgga cgagattgac
 721 ctgctgattc aggaaaccag ccgcctgctc cagctgaccc tcgagcatga ccctcacgat
 781 acactgggcc acgggctggg gcctgagacc agccccctga gctctccggc ttatactccc
 841 agcggggagg cgggcagctc tgcccggcag aaacctgtct tcgcaaggac ctgggtcgct
 901 ctgagtccct ccgcgtagtc tgccggccac accgcatctt ccggccgtcg gacctcatcc
 961 acggggaggt gctgggcaag ggctgcttcg gccaggctat caaggtgaca caccgtgaga
1021 caggtgaggt gatggtgatg aaggagctga tccggttcga cgaggagacc cagaggacgt
1081 tcctcaagga ggtgaaggtc atgcgatgcc tggaacaccc caacgtgctc aagttcatcg
1141 gggtgctcta caaggacaag aggctcaact tcatcactga gtacatcaag ggcggcacgc
1201 tccggggcat catcaagagc atggacagcc agtacccatg gagccagaga gtgagctttg
1261 ccaaggacat cgcatcaggg atggcctacc tccactccat gaacatcatc caccgagacc
1321 tcaactccca caactgcctg gtccgcgaga acaagaatgt ggtggtggct gacttcgggc
1381 tggcgcgtct catggtggac gagaagactc agcctgaggg cctgcggagc ctcaagaagc
1441 cagaccgcaa gaagcgctac accgtggtgg gcaaccccta ctggatggca cctgagatga
1501 tcaacggccg cagctatgat gagaaggtgg atgtgttctc ctttgggatc gtcctgtgcg
1561 agatcatcgg gcgggtgaac gcagaccctg actacctgcc ccgcaccatg gactttggcc
1621 tcaacgtgcg aggattcctg gaccgctact gccccccaaa ctgccccccg agcttcttcc
1681 ccatcaccgt gcgctgttgc gatctggacc ccgagaagag gccatccttt gtgaagctgg
1741 aacactggct ggagaccctc cgcatgcacc tggccggcca cctgccactg ggcccacagc
1801 tggagcagct ggacagaggt ttctgggaga cctaccggcg cggcgagagc ggactgcctg
1861 cccaccctga ggtccccgac tga
```

SEQ ID NO:99

```
   1 atgcctggct tcgactacaa gttcctggag aagcccaagc gacggctgct gtgcccactg
  61 tgcgggaagc ccatgcgcga gcctgtgcag gtttccacct gcggccaccg tttctgcgat
 121 acctgcctgc aggagttcct cagtgaagga gtcttcaagt gccctgagga ccagcttcct
 181 ctggactatg ccaagatcta cccagacccg gagctggaag tacaagtatt gggcctgcct
 241 atccgctgca tccacagtga ggagggctgc cgctggagtg ggccactacg tcatctacag
 301 ggccacctga atacctgcag cttcaatgtc attccctgcc ctaatcgctg ccccatgaag
 361 ctgagccgcc gtgatctacc tgcacacttg cagcatgact gccccaagcg gcgcctcaag
 421 tgcgagtttt gtggctgtga cttcagtggg gaggcctatg aggtggatga gagttctctg
 481 ggctttggtt atcccaagtt catctcccac caggacattc gaaagcgaaa ctatgtgcgg
 541 gatgatgcag tcttcatccg tgctgctgtt gaactgcccc ggaagatcct cagctga
```

SEQ ID NO:104

```
   1 ttgcaggctg ctgggctggg gctaagggct gctcagtttc cttcagcggg gcactgggaa
  61 gcgccatggc actgcagggc atctcggtcg tggagctgtc cggcctggcc ccgggcccgt
 121 tctgtgctat ggtcctggct gacttcgggg cgcgtgtggt acgcgtggac cggcccggct
 181 cccgctacga cgtgagccgc ttgggccggg gcaagcgctc gctagtgctg gacctgaagc
 241 agccgcgggg agccgccgtg ctgcggcgtc tgtgcaagcg gtcggatgtg ctgctggagc
 301 ccttccgccg cggtgtcatg gagaaactcc agctgggccc agagattctg cagcgggaaa
 361 atccaaggct tatttatgcc aggctgagtg gatttggcca gtcaggaagc ttctgccggt
 421 tagctggcca cgatatcaac tatttggctt tgtcaggtgt tctctcaaaa attggcagaa
 481 gtggtgagaa tccgtatgcc ccgctgaatc tcctggctga ctttgctggt ggtggcctta
 541 tgtgtgcact gggcattata atggctcttt ttgaccgcac acgcactggc aagggtcagg
 601 tcattgatgc aaatatggtg gaaggaacag catatttaag ttctttttctg tggaaaactc
 661 agaaatcgag tctgtgggaa gcacctcgag gacagaacat gttggatggt ggagcacctt
 721 tctatacgac ttacaggaca gcagatgggg aattcatggc tgttggagca atagaacccc
 781 agttctacga gctgctgatc aaaggacttg gactaaagtc tgatgaactt cccaatcaga
 841 tgagcatgga tgattggcca gaaatgaaga agaagtttgc agatgtattt gcaaagaaga
 901 cgaaggcaga gtggtgtcaa atctttgacg gcacagatgc ctgtgtgact ccggttctga
 961 cttttgagga ggttgttcat catgatcaca acaaggaacg gggctcgttt atcaccagtg
1021 aggagcagga cgtgagcccc cgccctgcac ctctgctgtt aaacacccca gccatccctt
1081 ctttcaaaag ggatcctttc ataggagaac acactgagga gatacttgaa gaatttggat
```

Figure 10 (cont.)

```
1141 tcagccgcga agagatttat cagcttaact cagataaaat cattgaaagt aataaggtaa
1201 aagctagtct ctaacttcca ggcccacggc tcaagtgaat ttgaatactg catttacagt
1261 gtagagtaac acataacatt gtatgcatgg aaacatggag gaacagtatt acagtgtcct
1321 accactctaa tcaagaaaag aattacagac tctgattcta cagtgatgat tgaattctaa
1381 aaatggttat cattagggct tttgatttat aaaactttgg gtacttatac taaattatgg
1441 tagttattct gccttccagt ttgcttgata tatttgttga tattaagatt cttgacttat
1501 attttgaatg ggttctagtg aaaaaggaat gatatattct tgaagacatc gatatacatt
1561 tatttacact cttgattcta caatgtagaa aatgaggaaa tgccacaaat tgtatggtga
1621 taaaagtcac gtgaaacaga gtgattggtt gcatccaggc cttttgtctt ggtgttcatg
1681 atctccctct aagcacattc caaactttag caacagttat cacactttgt aatttgcaaa
1741 gaaaagtttc acctgtattg aatcagaatg ccttcaactg aaaaaaacat atccaaaata
1801 atgaggaaat gtgttggctc actacgtaga gtccagaggg acagtcagtt ttagggttgc
1861 ctgtatccag taactcgggg cctgtttccc cgtgggtctc tgggctgtca gctttccttt
1921 ctccatgtgt ttgatttctc ctcaggctgg tagcaagttc tggatcttat acccaacaca
1981 cagcaacatc cagaaataaa gatct
```

SEQ ID NO:114

```
   1 cggaggcgct gggcgcacgg cgcggagccg gccggagctc gaggccggcg gcggcgggag
  61 agcgacccgg gcggcctcgt agcggggccc cggatccccg agtggcggcc ggagcctcga
 121 aaagagattc tcagcgctga ttttgagatg atgggcttgg gaaacgggcg tcgcagcatg
 181 aagtcgccgc ccctcgtgct ggccgccctg gtggcctgca tcatcgtctt gggcttcaac
 241 tactggattg cgagctcccg gagcgtggac ctccagacac ggatcatgga gctggaaggc
 301 agggtccgca gggcggctgc agagagaggc gccgtggagc tgaagaagaa cgagttccag
 361 ggagagctgg agaagcagcg ggagcagctt gacaaaatcc agtccagcca caacttccag
 421 ctggagagcg tcaacaagct gtaccaggac gaaaaggcgg ttttggtgaa taacatcacc
 481 acaggtgaga ggctcatccg agtgctgcaa gaccagttaa agaccctgca gaggaattac
 541 ggcaggctgc agcaggatgt cctccagttt cagaagaacc agaccaacct ggagaggaag
 601 ttctcctacg acctgagcca gtgcatcaat cagatgaagg aggtgaagga acagtgtgag
 661 gagcgaatag aagaggtcac caaaaagggg aatgaagctg tagcttccag agacctgagt
 721 gaaaacaacg accagagaca gcagctccaa gccctcagtg agcctcagcc caggctgcag
 781 gcagcaggcc tgccacacac agaggtgcca caagggaagg gaaacgtgct tggtaacagc
 841 aagtcccaga caccagcccc cagttccgaa gtggttttgg attcaaagag acaagttgag
 901 aaagaggaaa ccaatgagat ccaggtggtg aatgaggagc ctcagaggga caggctgccg
 961 caggagccag gccgggagca ggtggtggaa gacagacctg taggtggaag aggcttcggg
1021 ggagccggag aactgggcca gaccccacag gtgcaggctg ccctgtcagt gagccaggaa
1081 aatccagaga tggagggccc tgagcgagac cagcttgtca tccccgacgg acaggaggag
1141 gagcaggaag ctgccgggga agggagaaac cagcagaaac tgagaggaga agatgactac
1201 aacatggatg aaaatgaagc agaatctgag acagacaagc aagcagccct ggcagggaat
1261 gacagaaaca tagatgtttt taatgttgaa gatcagaaaa gagacaccat aaatttactt
1321 gatcagcgtg aaaagcggaa tcatacactc tgaattgaac tggaatcaca tatttcacaa
1381 cagggccgaa gagatgacta taaaatgttc atgagggact gaatactgaa aactgtgaaa
1441 tgtactaaat aaaatgtaca tctgaagatg attattgtga aattttagta tgcactttgt
1501 gtaggaaaaa atggaatggt cttttaaaca gcttttgggg gggtactttg gaagtgtcta
1561 ataaggtgtc acaatttttg gtagtaggta tttcgtgaga agttcaacac caaaactgga
1621 acatagttct ccttcaagtg ttggcgacag cggggcttcc tgattctgga atataacttt
1681 gtgtaaatta acagccacct atagaagagt ccatctgctg tgaaggagag acagagaact
1741 ctgggttccg tcgtcctgtc cacgtgctgt accaagtgct ggtgccagcc tgttacctgt
1801 tctcactgaa aagtctggct aatgctcttg tgtagtcact tctgattctg acaatcaatc
1861 aatcaatggc ctagagcact gactgttaac acaaacgtca ctagcaaagt agcaacagct
1921 ttaagtctaa atacaaagct gttctgtgtg agaatttttt aaaaggctac ttgtataata
1981 acccttgtca tttttaatgt acaaaacgct attaagtggc ttagaatttg aacatttgtg
2041 gtctttattt actttgcttc gtgtgtgggc aaagcaacat cttccctaaa tatatattac
2101 caagaaaagc aagaagcaga ttaggttttt gacaaaacaa acaggccaaa aggggctga
2161 cctggagcag agcatggtga gaggcaaggc atgagagggc aagtttgttg tggacagatc
```

Figure 10 (cont.)

```
2221 tgtgcctact ttattactgg agtaaaagaa aacaaagttc attgatgtcg aaggatatat
2281 acagtgttag aaattaggac tgtttagaaa aacaggaata caatggttgt ttttatcata
2341 gtgtacacat ttagcttgtg gtaaatgact cacaaaactg attttaaaat caagttaatg
2401 tgaattttga aaattactac ttaatcctaa ttcacaataa caatggcatt aaggtttgac
2461 ttgagttggt tcttagtatt atttatggta aataggctct taccacttgc aaataactgg
2521 ccacatcatt aatgactgac ttcccagtaa ggctctctaa ggggtaagta ggaggatcca
2581 caggatttga gatgctaagg ccccagagat cgtttgatcc aaccctctta ttttcagagg
2641 ggaaaatggg gcctagaagt tacagagcat ctagctggtg cgctggcacc cctggcctca
2701 cacagactcc cgagtagctg ggactacagg cacacagtca ctgaagcagg ccctgtttgc
2761 aattcacgtt gccacctcca acttaaacat tcttcatatg tgatgtcctt agtcactaag
2821 gttaaacttt cccacccaga aaaggcaact tagataaaat cttagagtac tttcatactc
2881 ttctaagtcc tcttccagcc tcactttgag tcctccttgg ggttgatagg aattttctct
2941 tgctttctca ataaagtctc tattcatctc atgtttaatt tgtacgcata gaattgctga
3001 gaaataaaat gttctgttca acttaaaaaa aaaaaaaaaa aa
```

SEQ ID NO:115

```
   1 cgggcgatgc cgcgctgcgg gggggccgca cagccgccgc caccgccacc gccgccgggt
  61 ggggtgggag gggcgggaac gcgcgccgcc gcctccaggg tgggcgcctt tcgccgtgga
 121 cgccgaccgt ccgggacgag ggtttcatca ccttaaatgg ttttgaacca atgaaggtgt
 181 attcccttaa aaagacggac agcccatcgt gtgaactata gagtttgtgg acagatttat
 241 attgggttca tagtggcgtc atgcacgcag actcctgcaa gttcccctaa gttcttagag
 301 gactgctttg ccttttgatc tgagagttgc aaagttccat aaagaatggc ccttgtggat
 361 aagcacaaag tcaagagaca gcgattggac agaatttgtg aaggtatccg cccccagatc
 421 atgaacggcc ccctgcaccc ccgccccctg gtggcgctgc tggacggccg cgactgcact
 481 gtggagatgc ccatcctgaa ggacctggcc actgtggcct tctgtgacgc gcagtcgacg
 541 caggaaatcc acgagaaggt tctaaacgaa gccgtgggcg ccatgatgta ccacaccatc
 601 accctcacca gggaggacct ggagaagttc aaggccctga gagtgatcgt gcggataggc
 661 agtggctatg acaacgtgga catcaaggct gccggcgagc tcggaattgc cgtgtgcaac
 721 atcccgtctg cagccgtgga agagacagcg gactctacca tctgccacat cctcaacctg
 781 taccggagga acacgtggct gtaccaggca ctgcgggaag gcacgcgggt tcagagcgtg
 841 gagcagatcc gcgaggtggc ctcgggagcg gcccgcatcc gtggggagac gctgggcctc
 901 attggctttg gtcgcacggg gcaggcggtt gcagttcgag ccaaggcctt tggattcagc
 961 gtcatatttt atgaccccta cttgcaggat gggatcgagc ggtccctggg cgtgcagagg
1021 gtctacaccc tgcaggattt gctgtatcag agcgactgcg tctccttgca ctgcaatctc
1081 aacgaacata accaccacct catcaatgac tttaccataa agcagatgag gcagggagca
1141 ttccttgtga acgcagcccg tggcggcctg gtggacgaga aagccttagc acaagccctc
1201 aaggagggca ggatacgagg ggcagccctc gacgtgcatg agtcagagcc cttcagcttt
1261 gctcagggtc cgttgaaaga tgccccgaat ctcatctgca ctcctcacac tgcctggtac
1321 agtgagcagg cgtcactgga gatgagggag gcagctgcca ccgagatccg ccgagccatc
1381 acaggtcgca tcccagaaag cttaagaaat tgtgtgaaca aggaattctt tgtcacatca
1441 gcgccttggt cagtaataga ccagcaagca attcatcctg agctcaatgg tgccacatac
1501 agatatccgc caggcatcgt gggtgtggct ccaggaggac ttcctgcagc catggaaggg
1561 atcatccctg gaggcatccc agtgactcac aacctcccga cagtggcaca tccttcccaa
1621 gcgccctctc ccaaccagcc cacaaaacac ggggacaatc gagagcaccc caacgagcaa
1681 tagcagagaa tgccagaagg taatcactca gatacacttg ggaccaagag acagtgaaaa
1741 atagatgaac taagagaaaa agaatcggat ggtctttgta actgattctg gacatatgca
1801 tcattgatgt tgcagtgttg aaactacaag agctagaaaa ctgaagatgt cgtctgctta
1861 cggaagcgct gaaagactag gatgtgattt attaacgacc aacttctgtt attgtgtgtt
1921 aagtttttca tctgtgcatc aaatcacaaa aagaataaat agagcttttt cctttatcag
1981 tcccttgggc acagcaggtc ctgaacaccc tgctctacaa tgttgcatca agagttcaaa
2041 caacaaaata aaaaatatta agaggaaatc cccatcctgt gacttgagtc ccttaagtct
2101 acaggggctg gtgacctctt tttgctaata ggaaaatcac attactacaa aatggggaga
```

Figure 10 (cont.)

```
2161 aaactgtttg cctgtggtag acacctgcac gcataggatt gaagacagta caggctgctg
2221 tacagagaag cgcctctcac atctgaactg catactgagc gggcaagtcg gttgtaagtt
2281 cagtaaaacc ctctgatgat gcaaaaaaaa aaaaaaagta ttaagtttca caagctgttt
2341 gtactcaaat atattttctc agtttcag
```

SEQ ID NO:116

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   1 catttgggga cgctctcagc tctcggcgca cggcccagct tccttcaaaa tgtctactgt
  61 tcacgaaatc ctgtgcaagc tcagcttgga gggtgatcac tctacacccc caagtgcata
 121 tgggtctgtc aaagcctata ctaactttga tgctgagcgg gatgctttga acattgaaac
 181 agccatcaag accaaaggtg tggatgaggt caccattgtc aacattttga ccaaccgcag
 241 caatgcacag agacaggata ttgccttcgc ctaccagaga aggaccaaaa aggaacttgc
 301 atcagcactg aagtcagcct tatctggcca cctggagacg gtgattttgg gcctattgaa
 361 gacacctgct cagtatgacg cttctgagct aaaagcttcc atgaaggggc tgggaaccga
 421 cgaggactct ctcattgaga tcatctgctc cagaaccaac caggagctgc aggaaattaa
 481 cagagtctac aaggaaatgt acaagactga tctggagaag gacattattt cggacacatc
 541 tggtgacttc cgcaagctga tggttgccct ggcaaagggt agaagagcag aggatggctc
 601 tgtcattgat tatgaactga ttgaccaaga tgctcgggat ctctatgacg ctggagtgaa
 661 gaggaaagga actgatgttc ccaagtggat cagcatcatg accgagcgga gcgtgcccca
 721 cctccagaaa gtatttgata ggtacaagag ttacagccct tatgacatgt tggaaagcat
 781 caggaaagag gttaaaggag acctggaaaa tgctttcctg aacctggttc agtgcattca
 841 gaacaagccc ctgtattttg ctgatcggct gtatgactcc atgaagggca aggggacgcg
 901 agataaggtc ctgatcagaa tcatggtctc ccgcagtgaa gtggacatgt tgaaaattag
 961 gtctgaattc aagagaaagt acggcaagtc cctgtactat tatatccagc aagacactaa
1021 gggcgactac cagaaagcgc tgctgtacct gtgtggtgga gatgactgaa gcccgacacg
1081 gcctgagcgt ccagaaatgg tgctcaccat gcttccagct aacaggtcta gaaaaccagc
1141 ttgcgaataa cagtccccgt ggccatccct gtgagggtga cgttagcatt acccccaacc
1201 tcattttagt tgcctaagca ttgcctggcc ttcctgtcta gtctctcctg taagccaaag
1261 aaatgaacat tccaaggagt tggaagtgaa gtctatgatg tgaaacactt tgcctcctgt
1321 gtactgtgtc ataaacagat gaataaactg aatttgtact tt
```

SEQ ID NO:117

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   1 gccccaggtg cgcttcccct agagagggat tttccggtct cgtgggcaga ggaacaacca
  61 ggaacttggg ctcagtctcc accccacagt ggggcggatc cgtcccggat aagacccgct
 121 gtctggccct gagtagggtg tgacctccgc agccgcagag gaggagcgca gcccggcctc
 181 gaagaacttc tgcttgggtg gctgaactct gatcttgacc tagagtcatg gccatggcaa
 241 ccaaaggagg tactgtcaaa gctgcttcag gattcaatgc catggaagat gcccagaccc
 301 tgaggaaggc catgaaaggg ctcggcaccg atgaagacgc cattattagc gtccttgcct
 361 accgcaacac cgcccagcgc caggagatca ggacagccta caagagcacc atcggcaggg
 421 acttgataga cgacctgaag tcagaactga gtggcaactt cgagcaggtg attgtgggga
 481 tgatgacgcc cacggtgctg tatgacgtgc aagagctgcg aagggccatg aagggagccg
 541 gcactgatga gggctgccta attgagatcc tggcctcccg gacccctgag gagatccggc
 601 gcataagcca aacctaccag cagcaatatg gacggagcct tgaagatgac attcgctctg
 661 acacatcgtt catgttccag cgagtgctgg tgtctctgtc agctggtggg agggatgaag
 721 gaaattatct ggacgatgct ctcgtgagac aggatgccca ggacctgtat gaggctggag
 781 agaagaaatg ggggacagat gaggtgaaat ttctaactgt tctctgttcc cggaaccgaa
 841 atcacctgtt gcatgtgttt gatgaataca aaaggatatc acagaaggat attgaacaga
 901 gtattaaatc tgaaacatct ggtagctttg aagatgctct gctggctata gtaaagtgca
 961 tgaggaacaa atctgcatat tttgctgaaa agctctataa atcgatgaag ggcttgggca
1021 ccgatgataa caccctcatc agagtgatgg tttctcgagc agaaattgac atgttggata
1081 tccgggcaca cttcaagaga ctctatggaa agtctctgta ctcgttcatc aagggtgaca
1141 catctggaga ctacaggaaa gtactgcttg ttctctgtgg aggagatgat taaaataaaa
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Figure 10 (cont.)

```
1201 atcccagaag gacaggagga ttctcaacac tttgaatttt tttaacttca ttttttctaca
1261 ctgctattat cattatctca gaatgcttat ttccaattaa aacgcctaca gctgcctcct
1321 agaatataga ctgtctgtat tattattcac ctataattag tcattatgat gctttaaagc
1381 tgtacttgca tttcaaagct tataagatat aaatggagat tttaaagtag aaataaatat
1441 gtattccatg tttttaaaag attactttct actttgtgtt tcacagacat tgaatatatt
1501 aaattattcc atattttctt ttcagtgaaa aatttttaa atggaagact gttctaaaat
1561 cacttttttc cctaatccaa tttttagagt ggctagtagt ttcttcattt gaaattgtaa
1621 gcatccggtc agtaagaatg cccatccagt tttctatatt tcatagtcaa agccttgaaa
1681 gcatctacaa atctcttttt ttaggttttg tccatagcat cagttgatcc ttactaagtt
1741 tttcatggga gacttccttc atcacatctt atgttgaaat cactttctgt agtcaaagta
1801 taccaaaacc aatttatctg aactaaattc taaagtatgg ttatacaaac catatacatc
1861 tggttaccaa acataaatgc tgaacattcc atattattat agttaatgtc ttaatccagc
1921 ttgcaagtga atggaaaaaa aaataagctt caaactaggt attctgggaa tgatgtaatg
1981 ctctgaattt agtatgatat aaagaaaact tttttgtgct aaaaatactt tttaaaatca
2041 attttgttga ttgtagtaat ttctatttgc actgtgcctt tcaactccag aaacattctg
2101 aagatgtact tggatttaat taaaaagttc actttgt
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SEQ ID NO:118

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  61 gggacccagc cctaggcaga acccaggcgc cgcgcccggg acgcccgcgg agagagccac
 121 tcccgcccac gtcccatttc gcccctcgcg tccggagtcc ccgtggccag atctaaccat
 181 gagctaccct ggctatcccc cgcccccagg tggctaccca ccagctgcac caggtggtgg
 241 tccctgggga ggtgctgcct accctcctcc gcccagcatg ccccccatcg ggctggataa
 301 cgtggccacc tatgcggggc agttcaacca ggactatctc tcgggaatgg cggccaacat
 361 gtctgggaca tttggaggag ccaacatgcc caacctgtac cctggggccc ctggggctgg
 421 ctacccacca gtgccccctg gcggctttgg gcagcccccc tctgcccagc agcctgttcc
 481 tccctatggg atgtatccac ccccaggagg aaacccaccc tccaggatgc cctcatatcc
 541 gccataccca ggggcccctg tgccgggcca gcccatgcca ccccccggac agcagccccc
 601 aggggcctac cctgggcagc caccagtgac ctaccctggt cagcctccag tgccactccc
 661 tgggcagcag cagccagtgc cgagctaccc aggatacccg gggtctggga ctgtcacccc
 721 cgctgtgccc ccaacccagt ttggaagccg aggcaccatc actgatgctc ccggctttga
 781 ccccctgcga gatgccgagg tcctgcggaa ggccatgaaa ggcttcggga cggatgagca
 841 ggccatcatt gactgcctgg ggagtcgctc caacaagcag cggcagcaga tcctactttc
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 961 ctttgagaag acaatcttgg ctctgatgaa gaccccagtc ctctttgaca tttatgagat
1021 aaaggaagcc atcaaggggg ttggcactga tgaagcctgc ctgattgaga tcctcgcttc
1081 ccgcagcaat gagcacatcc gagaattaaa cagagcctac aaagcagaat tcaaaaagac
1141 cctggaagag gccattcgaa gcgacacatc agggcacttc cagcggctcc tcatctctct
1201 ctctcaggga aaccgtgatg aaagcacaaa cgtggacatg tcactcgccc agagagatgc
1261 ccaggagctg tatgcggccg gggagaaccg cctgggaaca gacgagtcca agttcaatgc
1321 ggttctgtgc tcccggagcc gggcccacct ggtagcagtt ttcaatgagt accagagaat
1381 gacaggccgg gacattgaga agagcatctg ccgggagatg tccggggacc tggaggaggg
1441 catgctggcc gtggtgaaat gtctcaagaa taccccagcc ttctttgcgg agaggctcaa
1501 caaggccatg agggggcag gaacaaagga ccggaccctg attcgcatca tggtgtctcg
1561 cagcgagacc gacctcctgg acatcagatc agagtataag cggatgtacg gcaagtcgct
1621 gtaccacgac atctcgggag atacttcagg ggattaccgg aagattctgc tgaagatctg
1681 tggtggcaat gactgaacag tgactggtgg ctcacttctg cccacctgcc ggcaacacca
1741 gtgccaggaa aaggccaaaa gaatgtctgt ttctaacaaa tccacaaata gccccgagat
1801 tcaccgtcct agagcttagg cctgtcttcc acccctcctg acccgtatag tgtgccacag
1861 gacctgggtc ggtctagaac tctctcagga tgccttttct accccatccc tcacagcctc
1921 ttgctgctaa aatagatgtt tcatttttct gaaaaaaa
```

SEQ ID NO:119

Figure 10 (cont.)

```
   1 ggctcatgct cgggagcgtg gttgagcggc tggcgcggtt gtcctggagc aggggcgcag
  61 gaattctgat gtgaaactaa cagtctgtga gccctggaac ctccactcag agaagatgaa
 121 ggatatcgac ataggaaaag agtatatcat ccccagtcct gggtatagaa gtgtgaggga
 181 gagaaccagc acttctggga cgcacagaga ccgtgaagat tccaagttca ggagaactcg
 241 accgttggaa tgccaagatg ccttggaaac agcagcccga gccgagggcc tctctcttga
 301 tgcctccatg cattctcagc tcagaatcct ggatgaggag catcccaagg gaaagtacca
 361 tcatggcttg agtgctctga agcccatccg gactacttcc aaacaccagc acccagtgga
 421 caatgctggg ctttttttcct gtatgacttt ttcgtggctt tcttctctgg cccgtgtggc
 481 ccacaagaag ggggagctct caatggaaga cgtgtggtct ctgtccaagc acgagtcttc
 541 tgacgtgaac tgcagaagac tagagagact gtggcaagaa gagctgaatg aagttgggcc
 601 agacgctgct tccctgcgaa gggttgtgtg gatcttctgc cgcaccaggc tcatcctgtc
 661 catcgtgtgc ctgatgatca cgcagctggc tggcttcagt ggaccagcct tcatggtgaa
 721 acacctcttg gagtataccc aggcaacaga gtctaacctg cagtacagct tgttgttagt
 781 gctgggcctc ctcctgacgg aaatcgtgcg gtcttggtcg cttgcactga cttgggcatt
 841 gaattaccga accggtgtcc gcttgcgggg ggccatccta accatggcat ttaagaagat
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 961 cgatgggcag agaatgtttg aggcagcagc cgttggcagc ctgctggctg gaggacccgt
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1081 atcagctgtt tttatcctct tttacccagc aatgatgttt gcatcacggc tcacagcata
1141 tttcaggaga aaatgcgtgg ccgccacgga tgaacgtgtc cagaagatga atgaagttct
1201 tacttacatt aaatttatca aaatgtatgc ctgggtcaaa gcattttctc agagtgttca
1261 aaaaatccgc gaggaggagc gtcggatatt ggaaaaagct gggtacttcc agagcatcac
1321 tgtgggtgtg gctcccattg tggtggtgat tgccagcgtg gtgaccttct ctgttcatat
1381 gaccctgggc ttcgatctga cagcagcaca ggctttcaca gtggtgacag tcttcaattc
1441 catgactttt gctttgaaag taacaccgtt ttcagtaaag tccctctcag aagcctcagt
1501 ggctgttgac agatttaaga gtttgtttct aatggaagag gttcacatga taaagaacaa
1561 accagccagt cctcacatca agatagagat gaaaaatgcc accttggcat gggactcctc
1621 ccactccagt atccagaact cgcccaagct gacccccaaa atgaaaaaag acaagagggc
1681 ttccaggggc aagaaagaga aggtgaggca gctgcagcgc actgagcatc aggcggtgct
1741 ggcagagcag aaaggccacc tcctcctgga cagtgacgag cggcccagtc ccgaagagga
1801 agaaggcaag cacatccacc tgggccacct gcgcttacag aggacactgc acagcatcga
1861 tctggagatc caagagggta aactggttgg aatctgtggc agtgtgggaa gtggaaaaac
1921 ctctctcatt tcagccattt taggccagat gacgcttcta gagggcagca ttgcaatcag
1981 tggaaccttc gcttatgtgg cccagcaggc ctggatcctc aatgctactc tgagagacaa
2041 catcctgttt gggaaggaat atgatgaaga aagatacaac tctgtgctga acagctgctg
2101 cctgaggcct gacctggcca ttcttcccag cagcgacctg acggagattg gagagcgagg
2161 agccaacctg agcggtgggc agcgccagag gatcagcctt gcccgggcct tgtatagtga
2221 caggagcatc tacatcctgg acgacccccct cagtgcctta gatgcccatg tgggcaacca
2281 catcttcaat agtgctatcc ggaaacatct caagtccaag acagttctgt ttgttaccca
2341 ccagttacag tacctggttg actgtgatga agtgatcttc atgaaagagg gctgtattac
2401 ggaaagaggc acccatgagg aactgatgaa tttaaatggt gactatgcta ccatttttaa
2461 taacctgttg ctgggagaga caccgccagt tgagatcaat tcaaaaaagg aaaccagtgg
2521 ttcacagaag aagtcacaag acaagggtcc taaaacagga tcagtaaaga aggaaaaagc
2581 agtaaagcca gaggaagggc agcttgtgca gctggaagag aaagggcagg gttcagtgcc
2641 ctggtcagta tatggtgtct acatccaggc tgctgggggc cccttggcat tcctggttat
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2761 ctggatcaag caaggaagcg ggaacaccac tgtgactcga gggaacgaga cctcggtgag
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2941 agcttcctcc cggctgcatg acgagctttt ccgaaggatc cttcgaagcc ctatgaagtt
3001 ttttgacacg acccccacag ggaggattct caacaggttt tccaaagaca tggatgaagt
3061 tgacgtgcgg ctgccgttcc aggccgagat gttcatccag aacgttatcc tggtgttctt
3121 ctgtgtggga atgatcgcag gagtcttccc gtggttcctt gtggcagtgg ggcccctttgt
```

Figure 10 (cont.)

```
3181 catcctcttt tcagtcctgc acattgtctc cagggtcctg attcgggagc tgaagcgtct
3241 ggacaatatc acgcagtcac ctttcctctc ccacatcacg tccagcatac agggccttgc
3301 caccatccac gcctacaata aagggcagga gtttctgcac agataccagg agctgctgga
3361 tgacaaccaa gctccttttt ttttgtttac gtgtgcgatg cggtggctgg ctgtgcggct
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3481 gcagattccc ccagcctatg cgggtctcgc catctcttat gctgtccagt taacggggct
3541 gttccagttt acggtcagac tggcatctga gacagaagct cgattcacct cggtggagag
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3661 tccctccccc gactggcccc aggagggaga ggtgaccttt gagaacgcag agatgaggta
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3841 tctggtggag ttatctggag gctgcatcaa gattgatgga gtgagaatca gtgatattgg
3901 ccttgccgac ctccgaagca aactctctat cattcctcaa gagccggtgc tgttcagtgg
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4021 cctggagagg acacacatga aagaatgtat tgctcagcta cctctgaaac ttgaatctga
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4141 agccctgctc cgccactgta agattctgat tttagatgaa gccacagctg ccatggacac
4201 agagacagac ttattgattc aagagaccat ccgagaagca tttgcagact gtaccatgct
4261 gaccattgcc catcgcctgc acacggttct aggctccgat aggattatgg tgctggccca
4321 gggacaggtg gtggagtttg acaccccatc ggtccttctg tccaacgaca gttcccgatt
4381 ctatgccatg tttgctgctg cagagaacaa ggtcgctgtc aagggctgac tcctccctgt
4441 tgacgaagtc tcttttcttt agagcattgc cattccctgc ctggggcggg cccctcatcg
4501 cgtcctccta ccgaaacctt gcctttctcg attttatctt tcgcacagca gttccggatt
4561 ggcttgtgtg tttcactttt agggagagtc atattttgat tattgtattt attccatatt
4621 catgtaaaca aaatttagtt tttgttctta attgcactct aaaaggttca gggaaccgtt
4681 attataattg tatcagaggc ctataatgaa gctttatacg tgtagctata tctatatata
4741 attctgtaca tagcctatat ttacagtgaa aatgtaagct gtttatttta tattaaaata
4801 agcactgtgc taataacagt gcatattcct ttctatcatt tttgtacagt ttgctgtact
4861 agagatctgg ttttgctatt agactgtagg aagagtagca tttcattctt ctctagctgg
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4981 ctccgacagc cccctctgcc gcctccccac ggccgctcca ggggtggctg gagacgggtg
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5641 gctcaggtgg gcgtggtcac tgctgtcatc aattgaatgg tcagcgttgc atgtcgtgac
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5761 aaaatgtgaa taaaattatt ttggattttg t
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SEQ ID NO:120

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 121 ttttcttcaa tcacatctga ataaatcact tgaagaaagc ttatagcttc attgcaccat
 181 gtgtggcatt tgggcgctgt ttggcagtga tgattgcctt tctgttcagt gtctgagtgc
 241 tatgaagatt gcacacagag gtccagatgc attccgtttt gagaatgtca atggatacac
 301 caactgctgc tttggatttc accggttggc ggtagttgac ccgctgtttg gaatgcagcc
 361 aattcgagtg aagaaatatc cgtatttgtg gctctgttac aatggtgaaa tctacaacca
 421 taagaagatg caacagcatt ttgaatttga ataccagacc aaagtggatg gtgagataat
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Figure 10 (cont.)

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 601 agtcagacct ttgtttaaag caatgacaga agatggattt ttggctgtat gttcagaagc
 661 taaaggtctt gttacattga agcactccgc gactcccttt ttaaaagtgg agccttttct
 721 tcctggacac tatgaagttt tggatttaaa gccaaatggc aaagttgcat ccgtggaaat
 781 ggttaaatat catcactgtc gggatgtacc cctgcacgcc ctctatgaca atgtggagaa
 841 actctttcca ggttttgaga tagaaactgt gaagaacaac ctcaggatcc tttttaataa
 901 tgctgtaaag aaacgtttga tgacagacag aaggattggc tgccttttat caggggggctt
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1201 ttcagtaggt atgtatttaa tttccaagta tattcggaag aacacagata gcgtggtgat
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1321 ttctcctgaa aaagccgagg aggagagtga gaggcttctg agggaactct atttgtttga
1381 tgttctccgc gcagatcgaa ctactgctgc ccatggtctt gaactgagag tcccatttct
1441 agatcatcga ttttttttcct attacttgtc tctgccacca gaaatgagaa ttccaaagaa
1501 tgggatagaa aaacatctcc tgagagagac gtttgaggat tccaatctga tacccaaaga
1561 gattctctgg cgaccaaaag aagccttcag tgatggaata acttcagtta agaattcctg
1621 gtttaagatt ttacaggaat acgttgaaca tcaggttgat gatgcaatga tggcaaatgc
1681 agcccagaaa tttcccttca atactcctaa aaccaaagaa ggatattact accgtcaagt
1741 ctttgaacgc cattacccag gccgggctga ctggctgagc cattactgga tgcccaagtg
1801 gatcaatgcc actgaccctt ctgcccgcac gctgacccac tacaagtcag ctgtcaaagc
1861 ttaggtggtc tttatgctgt aatgtgaaag caaatatttc ttcgtgttgg atggggactg
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1981 attcctaaat tt
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SEQ ID NO:121

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 121 gtcaccatgg aagtgtcacc attgcagcct gtaaatgaaa atatgcaagt caacaaaata
 181 aagaaaaatg aagatgctaa gaaaagactg tctgttgaaa gaatctatca aaagaaaaca
 241 caattggaac atattttgct ccgcccagac acctacattg gttctgtgga attagtgacc
 301 cagcaaatgt gggtttacga tgaagatgtt ggcattaact atagggaagt cacttttgtt
 361 cctggtttgt acaaaatctt tgatgagatt ctagttaatg ctgcggacaa caaacaaagg
 421 gacccaaaaa tgtcttgtat tagagtcaca attgatccgg aaaacaattt aattagtata
 481 tggaataatg gaaaaggtat tcctgttgtt gaacacaaag ttgaaaagat gtatgtccca
 541 gctctcatat ttggacagct cctaacttct agtaactatg atgatgatga aaagaaagtg
 601 acaggtggtc gaaatggcta tggagccaaa ttgtgtaaca tattcagtac caaatttact
 661 gtggaaacag ccagtagaga atacaagaaa atgttcaaac agacatggat ggataatatg
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1321 atcaaagctg ccattggctg tggtattgta gaaagcatac taaactgggt gaagtttaag
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Figure 10 (cont.)

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1561 gacaaatatg gggttttccc tcttagagga aaaatactca atgttcgaga agcttctcat
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1681 aagaaaaact atgaagatga agattcattg aagacgcttc gttatgggaa gataatgatt
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1801 cacaactggc cctctcttct gcgacatcgt tttctggagg aatttatcac tcccattgta
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2041 aaatattctg gtcctgaaga tgatgctgct atcagcctgg cctttagcaa aaaacagata
2101 gatgatcgaa aggaatggtt aactaatttc atggaggata gaagacaacg aaagttactt
2161 gggcttcctg aggattactt gtatggacaa actaccacat atctgacata taatgacttc
2221 atcaacaagg aacttatctt gttctcaaat tctgataacg agagatctat cccttctatg
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4441 aaaggaacta aaagggatcc agctttgaat tctggtgtct ctcaaaagcc tgatcctgcc
4501 aaaaccaaga atcgccgcaa aaggaagcca tccacttctg atgattctga ctctaatttt
4561 gagaaaattg tttcgaaagc agtcacaagc aagaaatcca agggggagag tgatgacttc
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```

Figure 10 (cont.)

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5221 cagcctctta tgtgccaagt ttttctttaa gcaatgagaa attgctcatg ttcttcatct
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5461 gcagaagact cggggacaac atttgatcca agatcttaaa tgttatattg ataaccatgc
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5581 gttaagacct gtctacattg ttatatgtgt gtgacttgag taatgttatc aacgtttttg
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 121 acggggtcct tctgtgccag ctgctgcaca acctctcccc cggctccatc gacctcaagg
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 361 gcatcgcgca gaacaaaggg atcaggcctt ttccctcaga ggagaccaca gagaatgacg
 421 atgacgtcta ccgcagcctg gaggagctgg ccgacgagca tgacctgggg gaggacatct
 481 acgactgcgt cccgtgtgag gatggagggg acgacatcta cgaggacatc atcaaggtgg
 541 aggtgcagca gcccatgatt agatacatgc agaaaatggg catgactgaa gatgacaaga
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Figure 10 (cont.)

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2641 gacggcagga acgtggacaa gactcgcaga ttttcttggg agagtcactc cagccctgaa
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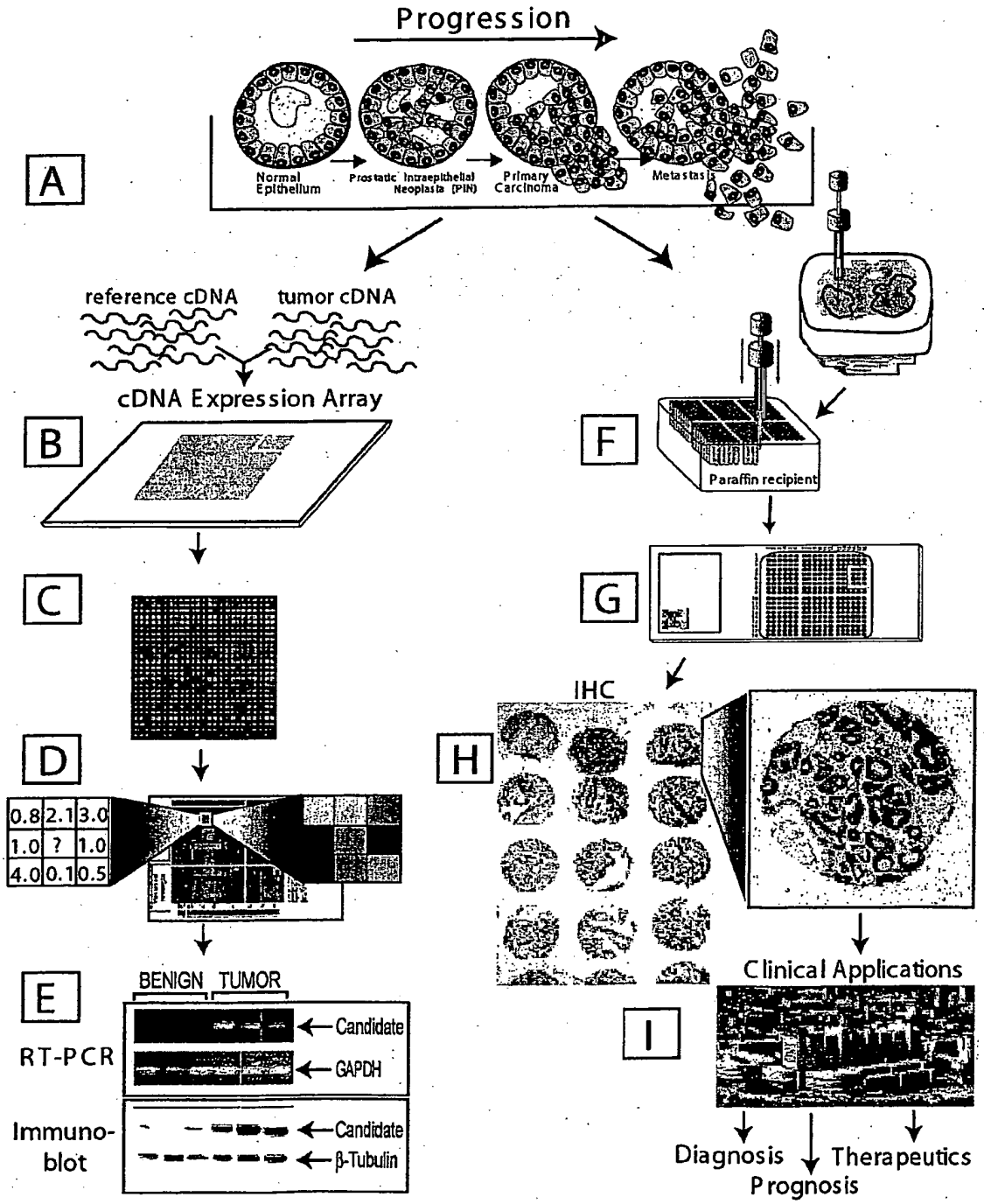
FIGURE 11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Normal Epithelium
Prostatic Intraepithelial Neoplasia (PIN)
Primary Carcinoma
Metastasis
reference cDNA
tumor cDNA
cDNA Expression Array
B
C
D
0.8 2.1 3.0
1.0 ? 1.0
4.0 0.1 0.5
E
BENIGN TUMOR
RT-PCR
Candidate
GAPDH
Immuno-blot
Candidate
β-Tubulin
F
Paraffin recipient
G
IHC
H
Clinical Applications
I
Diagnosis
Therapeutics
Prognosis

FIGURE 12

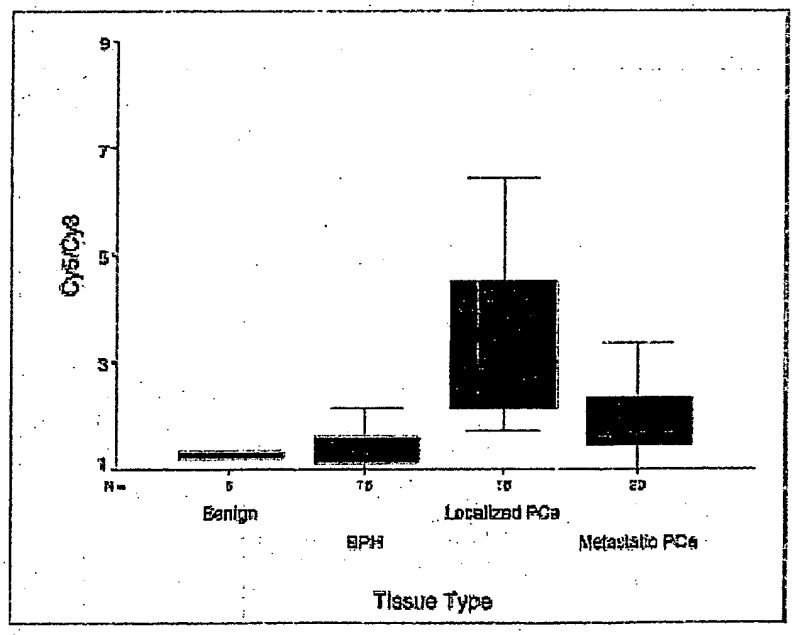
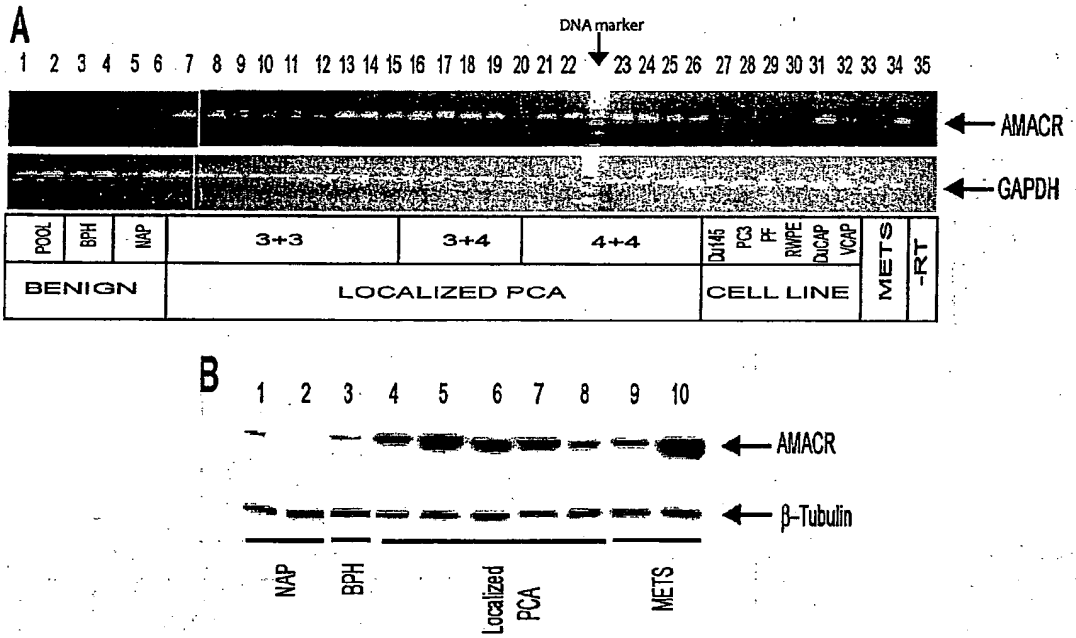

Cy5/Cy3
9
7
5
3
1
N=
5
75
15
20
Benign
BPH
Localized PCa
Metastatic PCa
Tissue Type

FIGURE 13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DNA marker
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
AMACR
GAPDH
POOL
BPH
NAP
3+3
3+4
4+4
Du145
PC3
PF
RWPE
DuCAP
VCAP
METS
-RT
BENIGN
LOCALIZED PCA
CELL LINE
B
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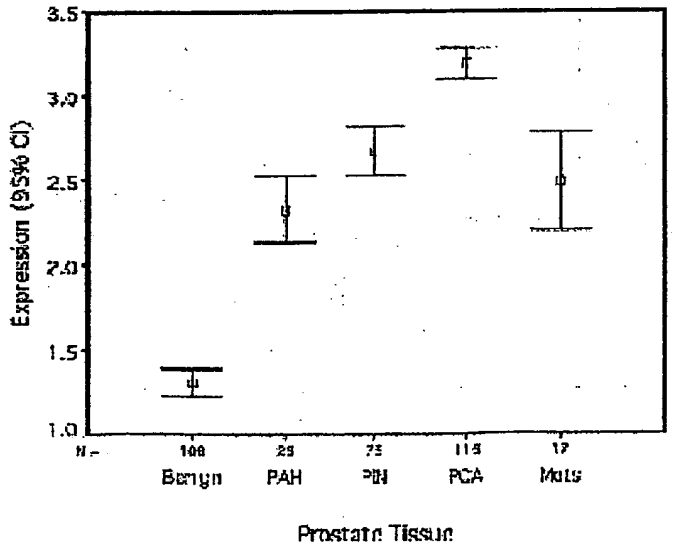
AMACR
β-Tubulin
NAP
BPH
Localized PCA
METS

FIGURE 14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3.0
2.5
2.0
1.5
1.0
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25
75
115
17
Benign
PAH
PIN
PCA
Mets
Prostate Tissue

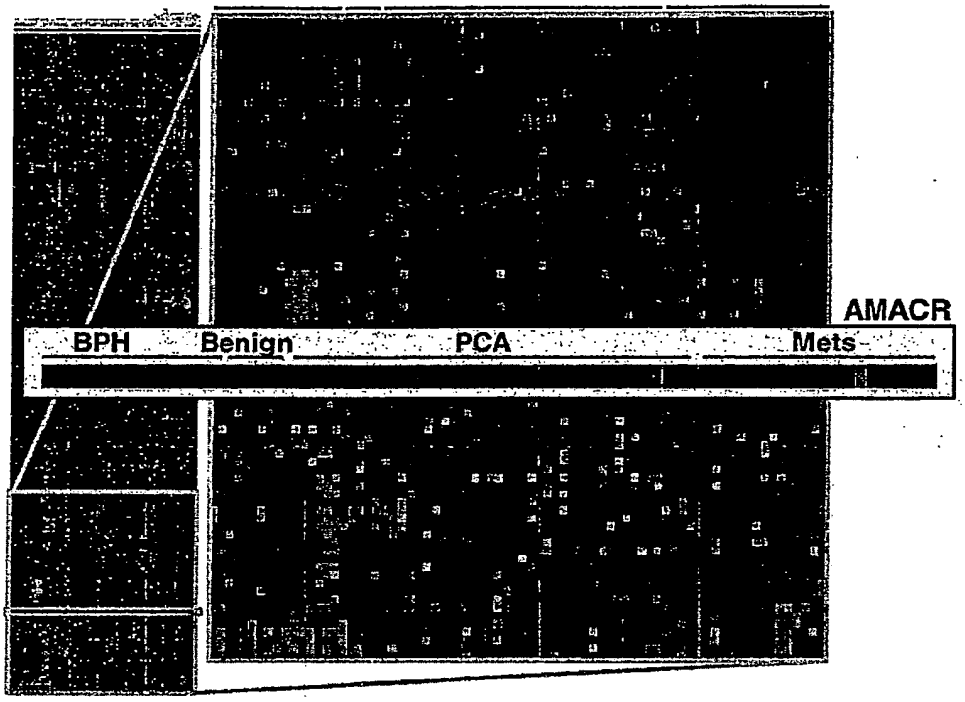
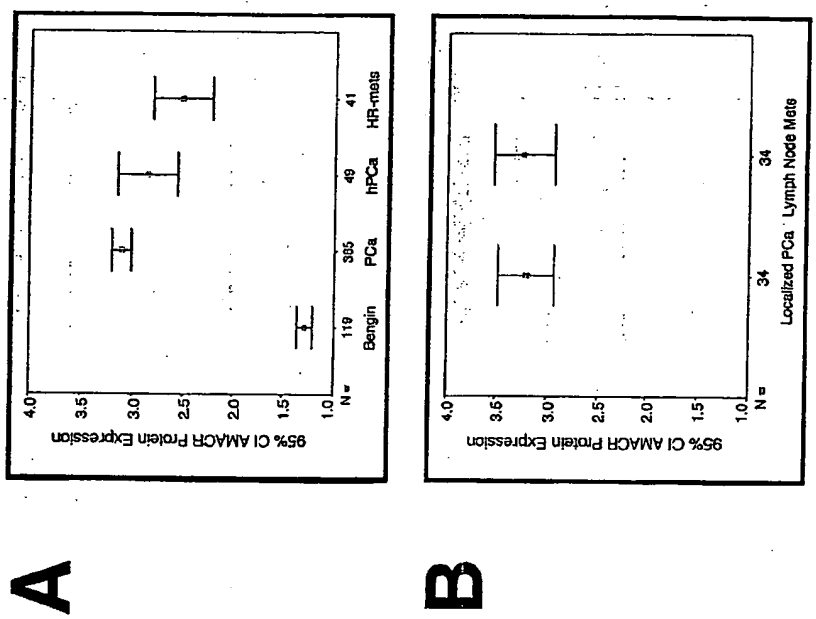


Figure 15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95% CI AMACR Protein Expression
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3.5
3.0
2.5
2.0
1.5
1.0
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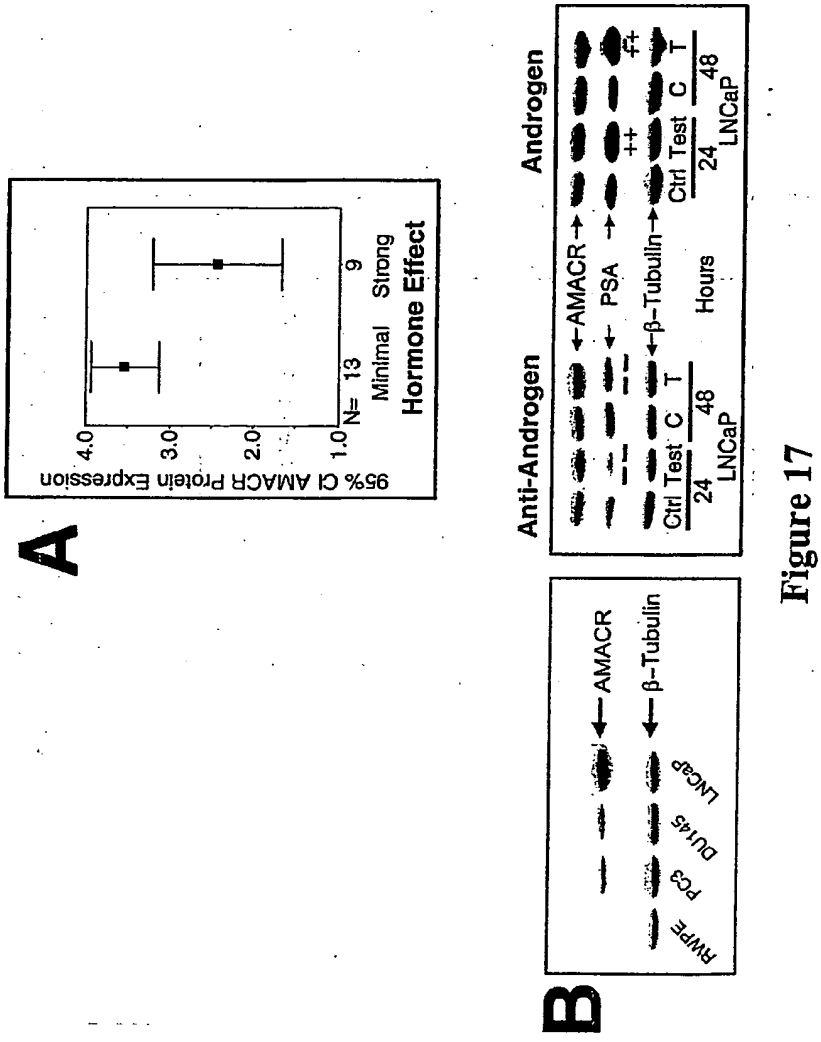
Benign
365
PCa
49
hPCa
41
HR-mets
B
95% CI AMACR Protein Expression
4.0
3.5
3.0
2.5
2.0
1.5
1.0
N =
34
Localized PCa
34
Lymph Node Mets

Figure 16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3.0
2.0
1.0
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Strong
Hormone Effect
B
RWPE
PC3
DU145
LNCaP
AMACR
β-Tubulin
Anti-Androgen
Androgen
AMACR
PSA
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Hours
Ctrl Test C T
24 48
LNCaP

Figure 17

Figure 18

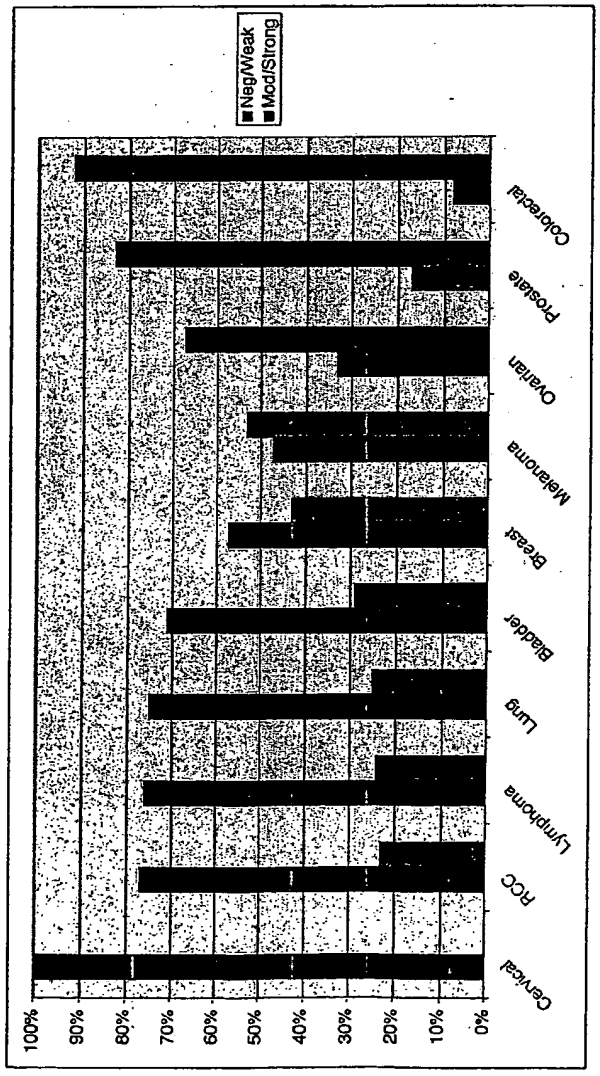

Neg/Weak
Mod/Strong
Cervical
RCC
Lymphoma
Lung
Bladder
Breast
Melanoma
Ovarian
Prostate
Colorectal
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%

Figure 19

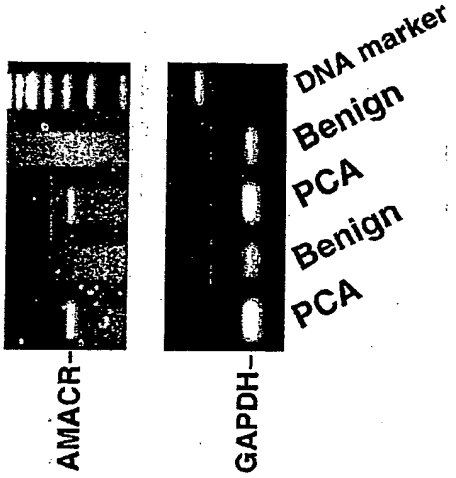


Figure 20

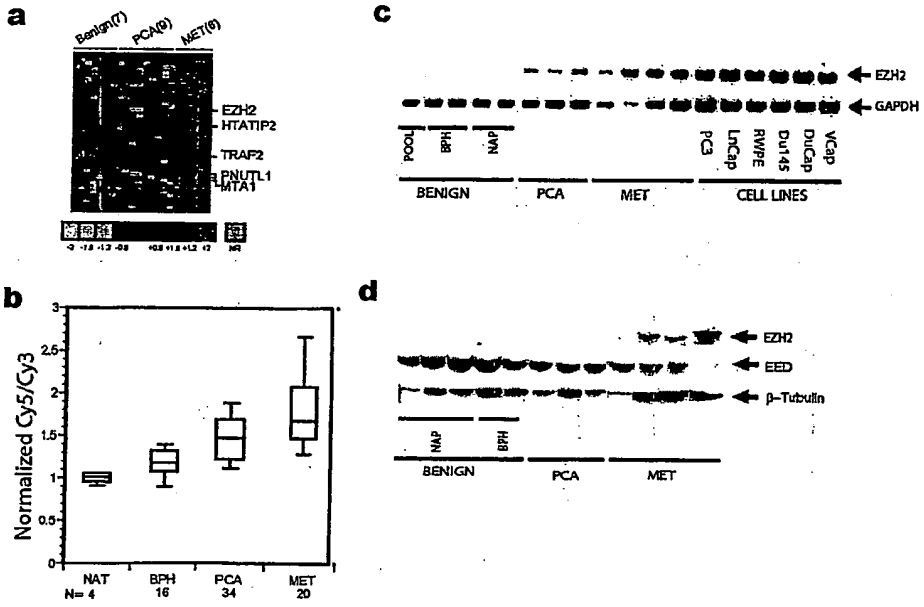

a
Benign(7)
PCA(9)
MET(9)
EZH2
HTATIP2
TRAF2
PNUTL1
MTA1
b
Normalized Cy5/Cy3
NAT
N= 4
BPH
16
PCA
34
MET
20
c
EZH2
GAPDH
PrSC
BPH
NAP
PC3
LnCap
RWPE
Du145
DuCap
vCap
BENIGN
PCA
MET
CELL LINES
d
EZH2
EED
β-Tubulin
NAP
BPH
BENIGN
PCA
MET

Figure 21

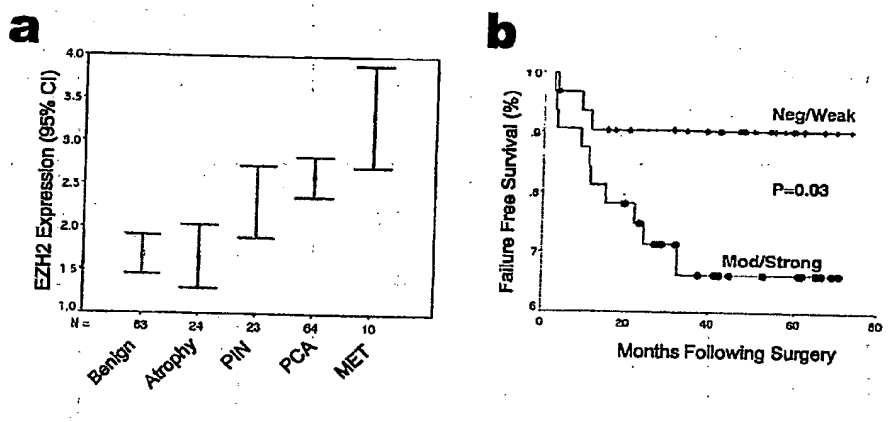

a
EZH2 Expression (95% CI)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
N =
63
24
23
64
10
Benign
Atrophy
PIN
PCA
MET
b
Failure Free Survival (%)
Neg/Weak
P=0.03
Mod/Strong
0
20
40
60
80
Months Following Surgery

Figure 22

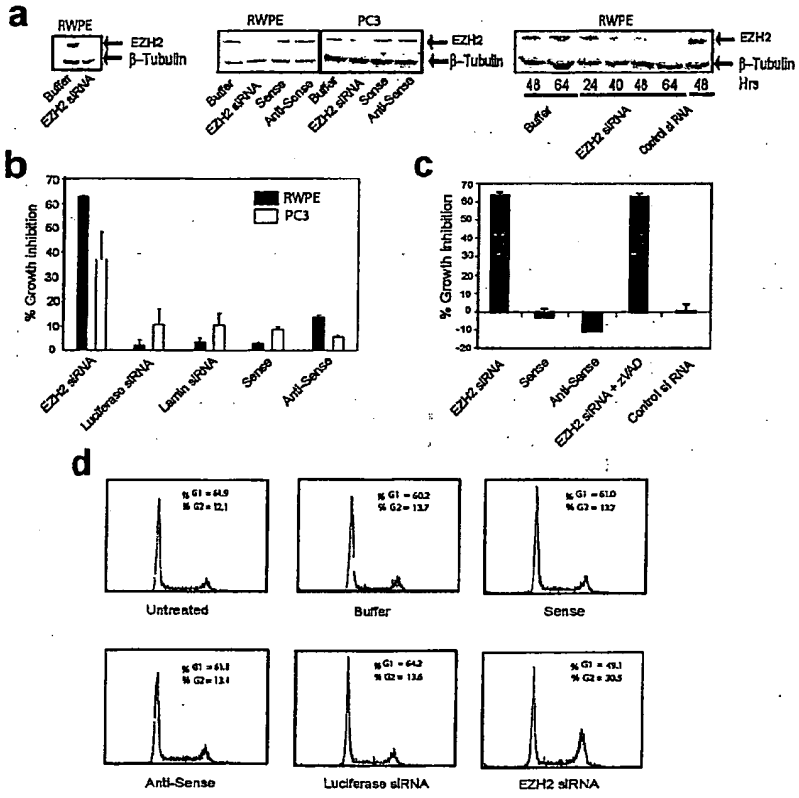

a
RWPE
EZH2
β-Tubulin
Buffer
EZH2 siRNA
RWPE
PC3
Buffer
EZH2 siRNA
Sense
Anti-Sense
RWPE
48 64 24 40 48 64 48
Hrs
Buffer
EZH2 siRNA
Control si RNA
b
% Growth Inhibition
RWPE
PC3
EZH2 siRNA
Luciferase siRNA
Lamin siRNA
Sense
Anti-Sense
c
% Growth Inhibition
EZH2 siRNA
Sense
Anti-Sense
EZH2 siRNA + zVAD
Control si RNA
d
Untreated
Buffer
Sense
Anti-Sense
Luciferase siRNA
EZH2 siRNA

Figure 23

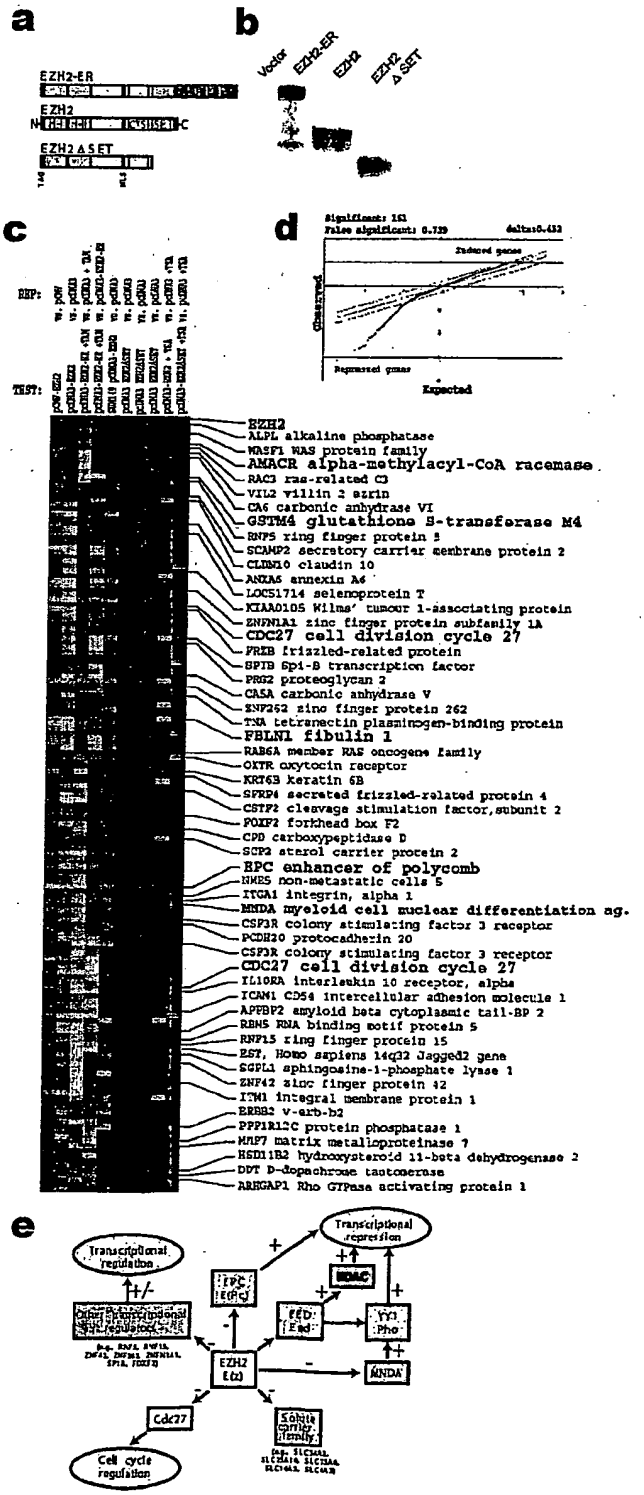

a
EZH2-ER
EZH2
EZH2 Δ SET
b
Vector
EZH2-ER
EZH2
EZH2 Δ SET
c
REP:
TEST:
d
Significant: 151
False significant: 0.739
delta=0.432
Induced genes
Observed
Repressed genes
Expected
EZH2
ALPL alkaline phosphatase
WASF1 WAS protein family
AMACR alpha-methylacyl-CoA racemase
RAC3 ras-related C3
VIL2 villin 2 ezrin
CA6 carbonic anhydrase VI
GSTM4 glutathione S-transferase M4
RNF5 ring finger protein 5
SCAMP2 secretory carrier membrane protein 2
CLDN10 claudin 10
ANXA5 annexin A5
LOC51714 selenoprotein T
KIAA0105 Wilms' tumour 1-associating protein
ZNFN1A1 zinc finger protein subfamily 1A
CDC27 cell division cycle 27
FRZB frizzled-related protein
SPIB Spi-B transcription factor
PRG2 proteoglycan 2
CA5A carbonic anhydrase V
ZNF262 zinc finger protein 262
TNA tetranectin plasminogen-binding protein
FBLN1 fibulin 1
RAB6A member RAS oncogene family
OXTR oxytocin receptor
KRT6B keratin 6B
SFRP4 secreted frizzled-related protein 4
CSTF2 cleavage stimulation factor,subunit 2
FOXF2 forkhead box F2
CPD carboxypeptidase D
SCP2 sterol carrier protein 2
EPC enhancer of polycomb
NME5 non-metastatic cells 5
ITGA1 integrin, alpha 1
MNDA myeloid cell nuclear differentiation ag.
CSF3R colony stimulating factor 3 receptor
PCDH20 protocadherin 20
CSF3R colony stimulating factor 3 receptor
CDC27 cell division cycle 27
IL10RA interleukin 10 receptor, alpha
ICAM1 CD54 intercellular adhesion molecule 1
APPBP2 amyloid beta cytoplasmic tail-BP 2
RBM5 RNA binding motif protein 5
RNF15 ring finger protein 15
E5T, Homo sapiens 14q32 Jagged2 gene
SGPL1 sphingosine-1-phosphate lyase 1
ZNF42 zinc finger protein 42
ITM1 integral membrane protein 1
ERBB2 v-erb-b2
PPP1R12C protein phosphatase 1
MMP7 matrix metalloproteinase 7
HSD11B2 hydroxysteroid 11-beta dehydrogenase 2
DDT D-dopachrome tautomerase
ARHGAP1 Rho GTPase activating protein 1
e
Transcriptional regulation
Transcriptional repression
EPC (EPC1)
HDAC
EED (Eed)
YY1 (Pho)
EZH2 (Ez)
MNDA
Cdc27
Cell cycle regulation

Figure 24
AMACR IN PCA CELL LINES

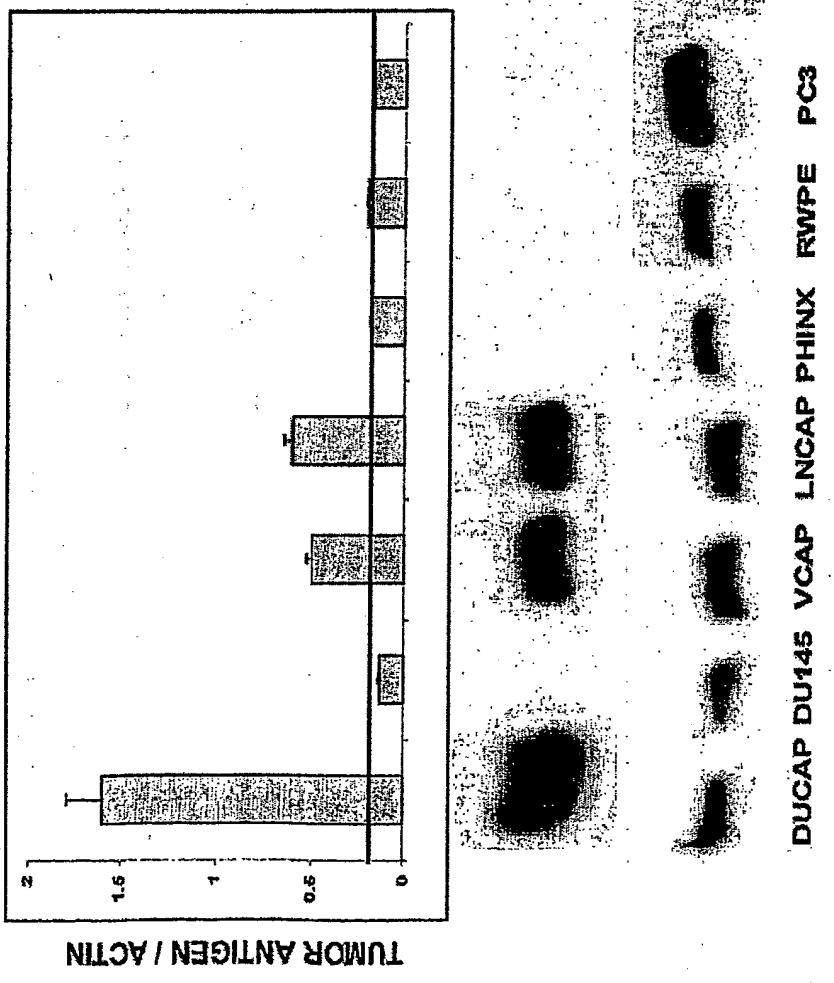


Figure 25
AMACR IN PCA SERA

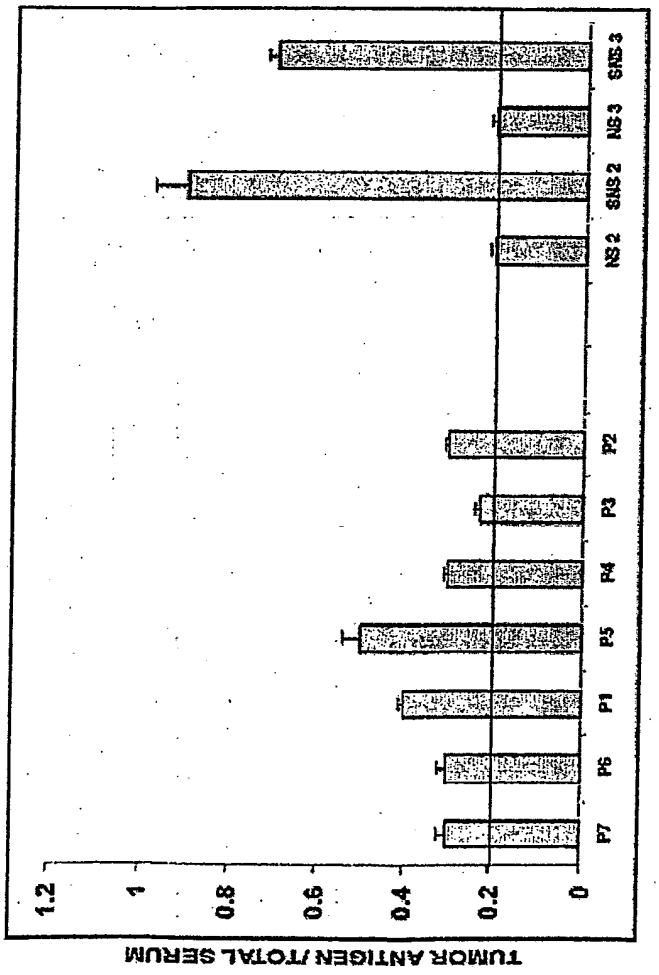
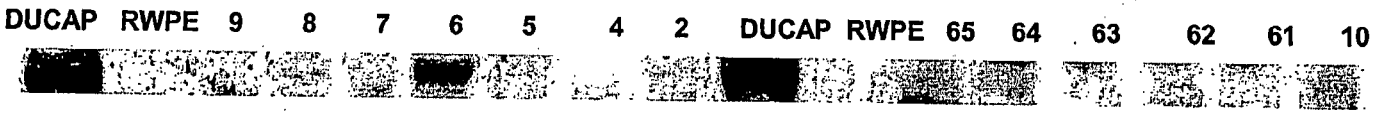
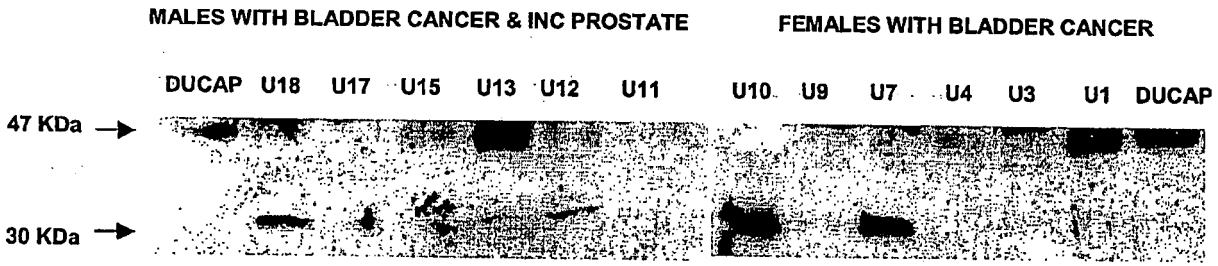


Figure 26
Immunoblot Analysis for AMACR in Prostate Cancer and Normal Sera



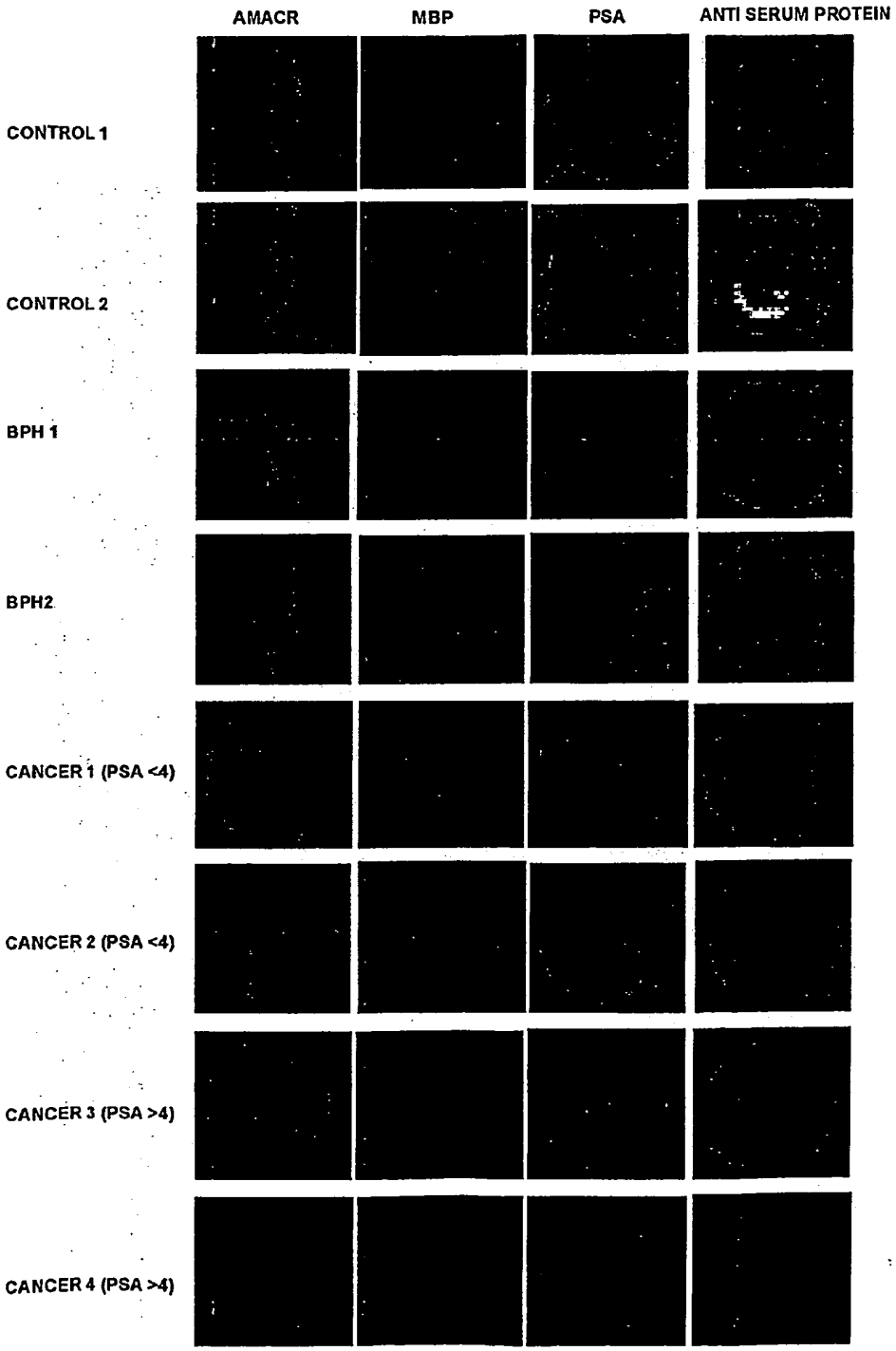
Sample	PSA
2-10	Positive
61-65	Negative

Figure 27
IMMUNOBLOT ANALYSIS OF URINE SAMPLES FOR AMACR



U1-U10 : FEMALES WITH BLADDER CANCER
U11-U20 : MALES WITH BLADDER CANCER AND INC PROSTATE

Figure 28


AMACR
MBP
PSA
ANTI SERUM PROTEIN
CONTROL 1
CONTROL 2
BPH 1
BPH2
CANCER 1 (PSA <4)
CANCER 2 (PSA <4)
CANCER 3 (PSA >4)
CANCER 4 (PSA >4)

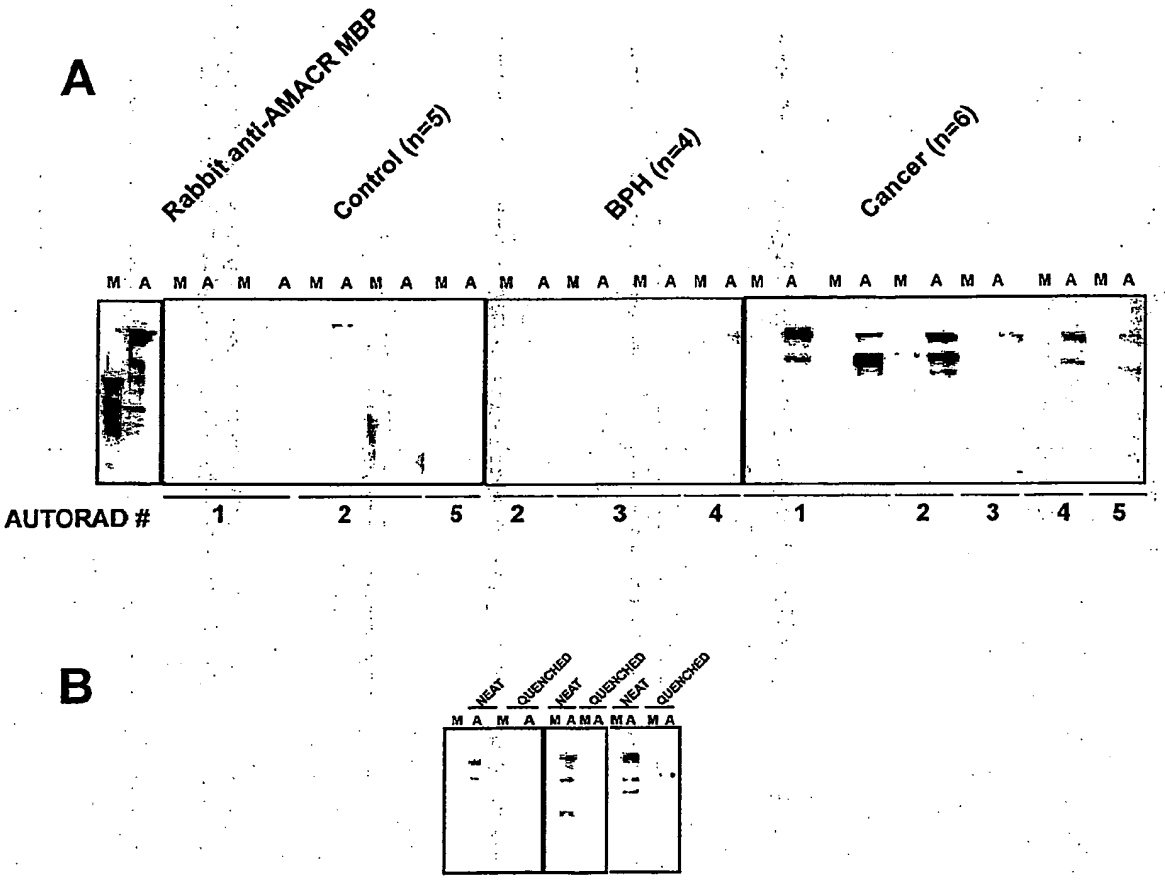

A
Rabbit anti-AMACR MBP
Control (n=5)
BPH (n=4)
Cancer (n=6)
M A M A M A M A M A M A M A M A M A M A M A M A M A M A M A M A M A
AUTORAD # 1 2 5 2 3 4 1 2 3 4 5
B
NEAT QUENCHED NEAT QUENCHED NEAT QUENCHED
M A M A M A M A M A M A

Figure 29

Figure 30

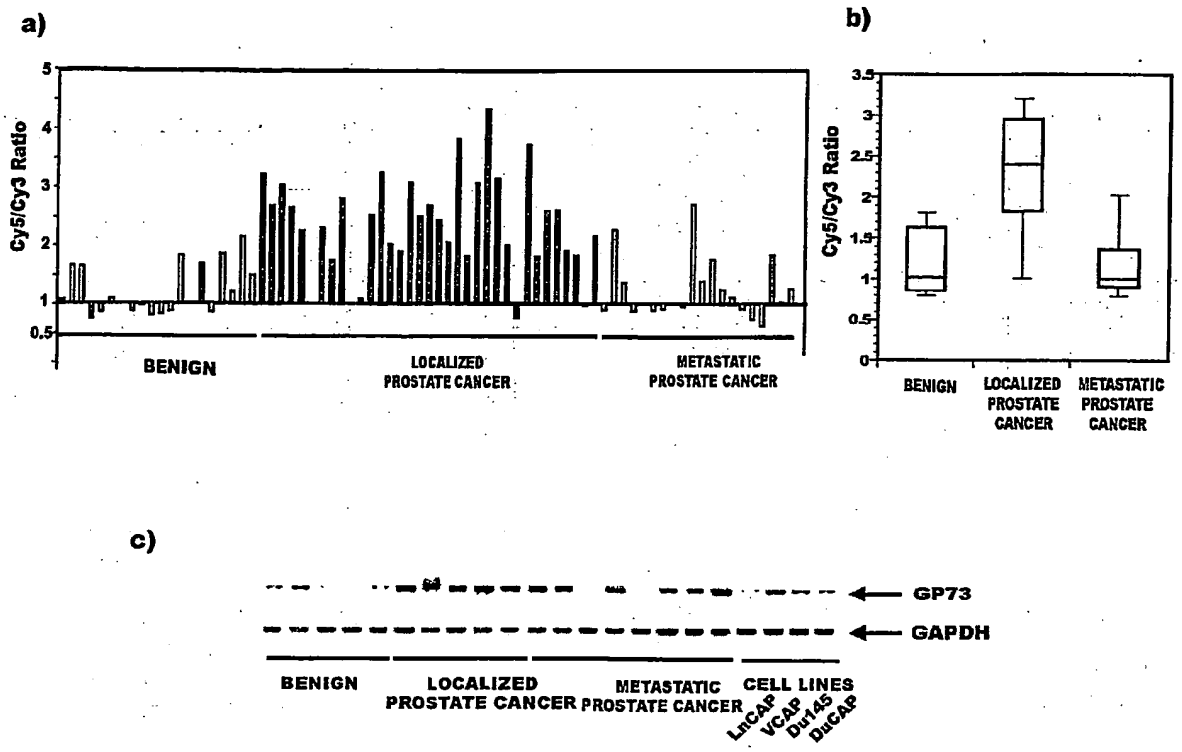

a)
Cy5/Cy3 Ratio
5
4
3
2
1
0.5
BENIGN
LOCALIZED PROSTATE CANCER
METASTATIC PROSTATE CANCER
b)
Cy5/Cy3 Ratio
3.5
3
2.5
2
1.5
1
0.5
0
BENIGN
LOCALIZED PROSTATE CANCER
METASTATIC PROSTATE CANCER
c)
GP73
GAPDH
BENIGN
LOCALIZED PROSTATE CANCER
METASTATIC PROSTATE CANCER
CELL LINES
LnCAP
VCAP
Du145
DuCAP

Figure 31

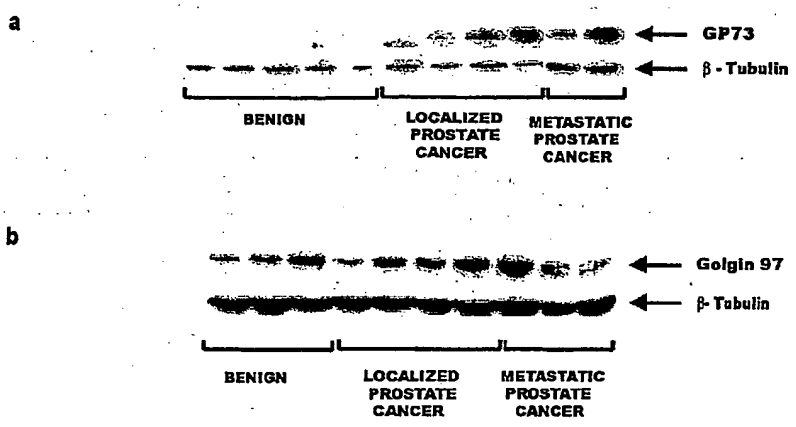

a
GP73
β - Tubulin
BENIGN
LOCALIZED PROSTATE CANCER
METASTATIC PROSTATE CANCER
b
Golgin 97
β- Tubulin
BENIGN
LOCALIZED PROSTATE CANCER
METASTATIC PROSTATE CANCER

Figure 32

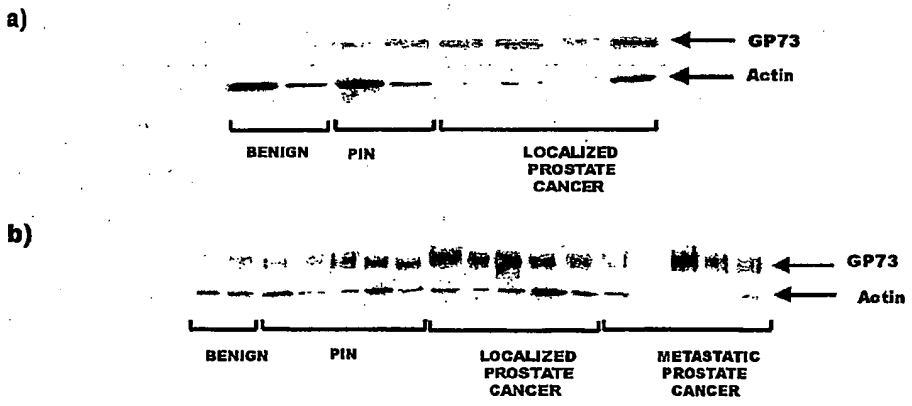
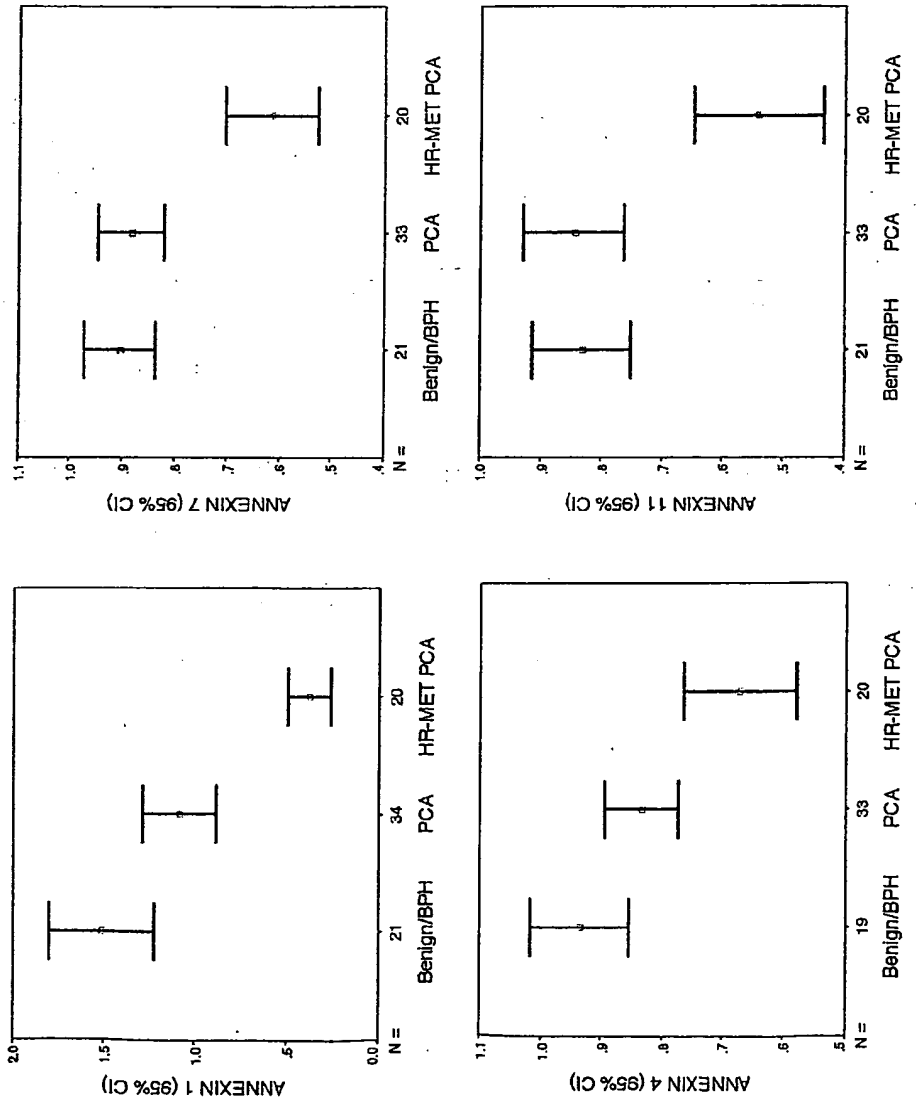

a)
GP73
Actin
BENIGN
PIN
LOCALIZED PROSTATE CANCER
b)
GP73
Actin
BENIGN
PIN
LOCALIZED PROSTATE CANCER
METASTATIC PROSTATE CANCER

Figure 33


ANNEXIN 1 (95% CI)
ANNEXIN 4 (95% CI)
ANNEXIN 7 (95% CI)
ANNEXIN 11 (95% CI)
N =
Benign/BPH
PCA
HR-MET PCA

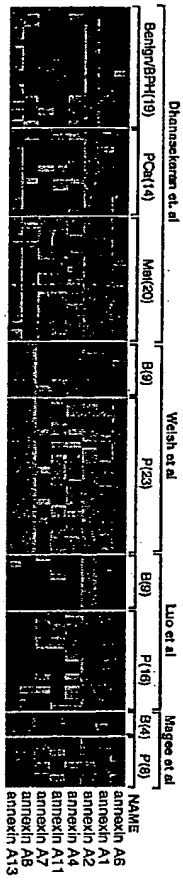

Dhanasekaran et al
Welsh et al
Luo et al
Magee et al
BenignBPH(19)
PCa(14)
Mal(20)
B(9)
P(23)
B(9)
P(16)
B(4)
P(8)
NAME
annexin A6
annexin A1
annexin A2
annexin A4
annexin A11
annexin A7
annexin A8
annexin A13

Figure 34

Figure 35

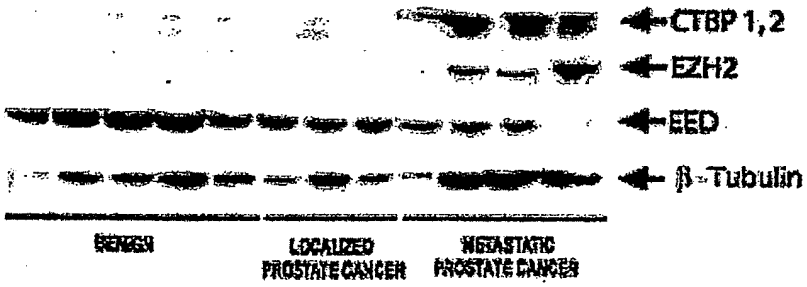

CTBP 1,2
EZH2
EED
β-Tubulin
BENIGN
LOCALIZED PROSTATE CANCER
METASTATIC PROSTATE CANCER

Figure 36

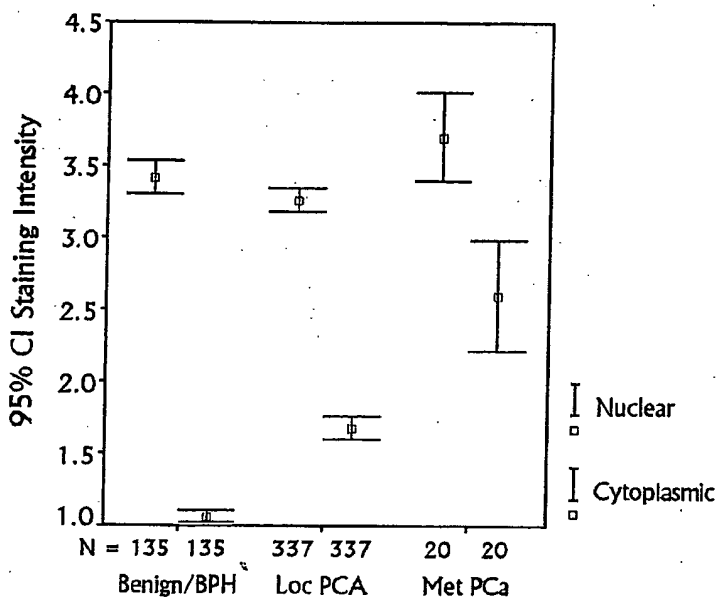

95% CI Staining Intensity
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
N = 135 135 337 337 20 20
Benign/BPH
Loc PCA
Met PCa
Nuclear
Cytoplasmic

Figure 37

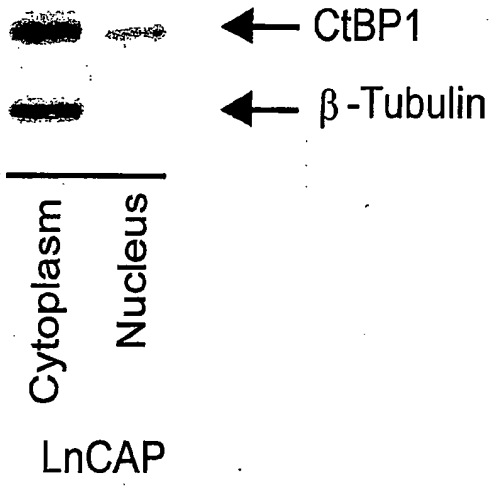
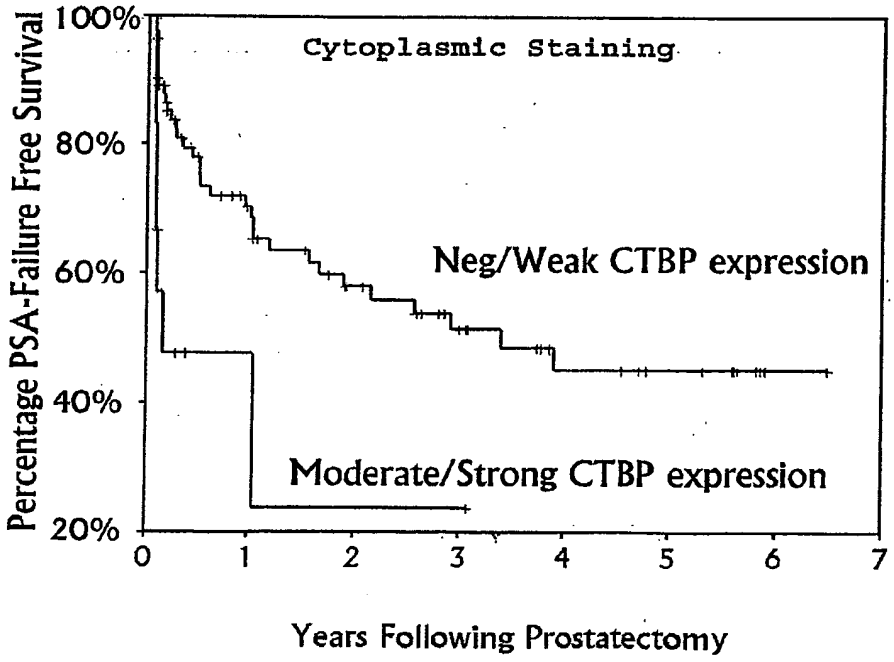


Figure 38


Cytoplasmic Staining
Neg/Weak CTBP expression
Moderate/Strong CTBP expression
Percentage PSA-Failure Free Survival
100%
80%
60%
40%
20%
0
1
2
3
4
5
6
7
Years Following Prostatectomy

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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PCT

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(26) Publication Language: English

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60/334,468	15 November 2001 (15.11.2001)	US
10/210,120	1 August 2002 (01.08.2002)	US

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(74) Agents: **ARENSON, Tanya, A.** et al.; Medlen & Carroll, LLP, 101 Howard Street, Suite 350, San Francisco, CA 94105 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 13 January 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: EXPRESSION PROFILE OF PROSTATE CANCER

(57) Abstract: The present invention relates to compositions and methods for cancer diagnostics, including but not limited to, cancer markers. In particular, the present invention provides gene expression profiles associated with prostate cancers. Genes identified as cancer markers using the methods of the present invention find use in the diagnosis and characterization of prostate cancer. In addition, the genes provide targets for cancer drug screens and therapeutic applications.

WO 2003/012067 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/24567

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/68; G01N 33/53; C07K 16/00; C07H 21/04
US CL : 435/6, 7.1; 530/387.1; 536/24.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 7.1; 530/387.1; 536/24.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,518,028 B1 (O'BRIEN) 11 February 2003 (11.02.03), claims 1-2.	1-3,6-7,9-10
---		---
Y		38-39, 41

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2004 (20.09.2004)	17 NOV 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Valerie Bell-Harris for MINH-TAM DAVIS Telephone No. 571-272-1600

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/24567

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13 and 38-41

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.